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Commercial Latex Agglutination Kits for the Detection of Foodborne *Salmonella*

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ABSTRACT

The ability of the Bactigen® *Salmonella Shigella* (BSST), the Microscreen® (MS), and the Spectate® (SPECT) latex agglutination kits to detect *Salmonella* in pure cultures and in naturally contaminated foods was examined. Of 190 *Salmonella* strains tested, the MS, BSST, and SPECT systems correctly identified 89.5, 81.6, and 66.3% of the test cultures, respectively. The sensitivity of SPECT increased to 92.7% when only strains belonging to the targeted serogroups (somatic A to E plus G) and strains harboring the Vi antigen were considered. The lack of specificity of the MS (3.4%), SPECT (17.0%), and BSST (33.9%) systems with 59 cultures of nonsalmonellae varied widely, with *Citrobacter freundii* and *Escherichia coli* accounting for many of the false-positive reactions. Examination of foods according to the prescribed MS and SPECT analytical test protocols identified respectively, 18 (75%) and 19 (79.2%) of the 24 food samples found to contain *Salmonella* spp. by a standard cultural method. Although instructions with the BSST kit indicate that the product is intended for the analysis of clinical samples, the system nevertheless identified 21 (87.5%) contaminated food samples under homologous MS and SPECT test conditions. The concurrent use of TBG_{43} with enrichment media recommended by kit manufactures enhanced the sensitivities of MS (83.3%), SPECT (91.7%), and BSST (91.7%). Attempts to effect greater method brevity through the application of latex kits at various stages of the standard cultural procedure were counterproductive.

Standard cultural methods for the detection of *Salmonella* in foods generally require four or more days to obtain presumptive evidence of product contamination. Such delays not only impede activities of regulatory agencies but also impact economically on the bacteriological clearance and release of stored ingredients and manufactured food products. The situation has in recent years encouraged major research efforts towards the development of more rapid and dependable diagnostic tools for the detection of foodborne salmonellae. Technological innovations have included the development and marketing of enzyme-linked immunosorbent (ELISA) kits *(3,6,11,15)*, self-contained immunoimmobilization assay vials *(7,14,20)*, chromogenic rRNA-specific DNA probes *(2,21)*, and impediometric *(9,19)* assay procedures. Most of these novel technologies provide presumptive evidence of foodborne *Salmonella* contamination one day earlier than with standard cultural methods. These rapid techniques, with the exception of impedance methods, rely on selective enrichment broth cultures or postenrichment cultures as test material *(4,6)*.

The present study examines the sensitivity and specificity of the commercial Microscreen® (MS, Mercia Diagnostics Limited, Surrey, UK), Spectate® (SPECT, May and Baker Diagnostics Ltd, Glasgow, UK) and the Bactigen® *Salmonella Shigella* (BSST, Wampole Laboratories, Cranbury, NJ) latex agglutination kits using pure cultures and naturally contaminated foods. The propensity for the more rapid detection of foodborne *Salmonella* through the application of these technologies at different stages in a standard cultural procedure was also investigated.

MATERIALS AND METHODS

Test cultures

A total of 190 *Salmonella* strains representing 27 somatic groups, as well as 59 nonsalmonellae strains (14 genera, 19 species) was examined in this study. Pure cultures of *Salmonella* and nonsalmonellae (Table 1) were obtained from our laboratory collection of microorganisms maintained at room temperature in sealed semisolid agar slants containing (per liter); meat extract (5 g); peptone (10 g); NaCl (3 g); Na_2HPO_4. 12 H_2O (2 g); agar (10 g); pH 7.4. Bacterial strains remain viable for several years in this medium.

Sample preparation for latex agglutination testing

Pure cultures. Stock cultures on semisolid agar were inoculated into 9 ml nutrient broth (NB) and incubated for 18 h at 35°C. A loopful of each broth culture was then subcultured into M broth and incubated for 18 h at 35°C. Cell densities in M broth cultures varied between 10^8 to 10^9 CFU/ml. Replicate portions of each M broth culture (one drop from Pasteur pipette or disposable droppers provided by BSST and SPECT) were tested with MS and BSST reagents according to manufacturer's instructions, whereas the Spectate assay relied on a replicate portion (1 ml) of the M broth culture previously held in a boiling water bath for 15-30 min.

Foods. Manufacturers specify that kit reagents be used for the presumptive identification of *Salmonella* spp. in M broth (MS) and NB (SPECT) postenrichment cultures (4-6 h at 35°C) follow-

TABLE 1. *Sensitivity and specificity of latex agglutination kits with pure cultures.*

Microorganism		No. strains tested	No. of positive latex reactions (%)		
			MS	BSST	SPECT
Salmonella spp					
Somatic group	A	1 (1)[a]	1	1	1
	B	25 (16)	25	24	24
	C	51 (39)	49	49	50
	D	21 (10)	17	21	21
	E	32 (21)	31	20	24
	F	4 (4)	4	1	0
	G	6 (5)	6	6	6
	H	4 (4)	4	3	0
	I	4 (3)	4	2	0
	Others[b]	42 (29)	29	28	0
	Subtotal	190 (132)	170 (89.5)	155 (81.6)	126 (66.3)[c]
Other *Enterobacteriaceae*					
Citrobacter freundii		14	1	11	7
Enterobacter aerogenes		1	0	1	1
Enterobacter agglomerans		4	0	2	0
Enterobacter cloacae		1	0	0	0
Escherichia coli		13	0	4	1
Hafnia alvei		1	0	0	0
Klebsiella pneumoniae		1	0	0	0
Morganella morganii		1	0	0	0
Proteus mirabilis		4	0	0	0
Proteus vulgaris		1	0	0	0
Providencia alcalifaciens		1	0	0	0
Providencia stuartii		1	0	0	0
Shigella sonnei		1	0	0	0
Yersinia enterocolitica		2	0	0	0
Nonrelated genera					
Achromobacter xylosoxidans		1	0	0	0
Aeromonas hydrophila		9	1	1	1
Moraxella osloensis		1	0	0	0
Pseudomonas aeruginosa		1	0	0	0
Staphylococcus aureus		1	0	1	0
	Subtotal	59	2 (3.4)	20 (33.9%)	10 (17.0%)

[a]Numbers in brackets denote corresponding number of serovars.
[b]Relates to 18 additional somatic serogroups.
[c]Spectate sensitivity for intended serogroups (136 test strains) was 92.7%.

ing the sequential preenrichment and selective enrichment of food samples for 18 h each at 35°C. Suspect colonies on differential agar media can also be used as test material in the SPECT assay. In contrast, prescribed conditions of use of BSST reagents focus on clinical test materials such as those arising from the direct enrichment or direct plating of stools. Package inserts bore no reference to BSST-dependent detection of foodborne *Salmonella*. In the present study, the potential for greater method brevity through the application of these novel diagnostic kits at various stages of a standard cultural procedure was examined (Fig. 1). Briefly, representative portions (one drop from Pasteur pipette or disposable droppers provided by manufacturers) of each preenrichment and enrichment cultures (6 and 24 h) were assayed directly with the MS and BSST reagents. SPECT reagents were applied to replicates of the same broth cultures previously held in a boiling water bath for 15-30 min. Portions (1-ml) of each 24 h enrichment culture were also postenriched in nine volumes of M

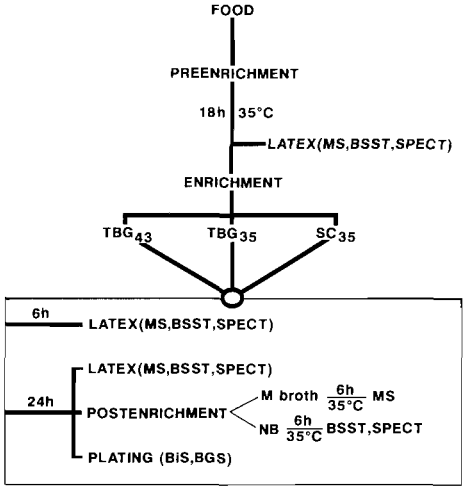

Figure 1. *Detection of foodborne* Salmonella *by latex agglutination kits.*

(MS) or NB (BSST, SPECT) broths for 6 h at 35°C; postenrichment cultures were then tested with appropriate latex kit reagents (Fig.1).

Latex agglutination assays

Latex beads with bound *Salmonella*-specific antibodies amplify agglutination reactions and expedite visual identification of positive samples. Reagents of the three latex agglutination kits examined in the present study respond to *Salmonella* flagellar (MS) or somatic (BSST, SPECT) antigens. Agglutination reactions were carried out on prescored test cards with black (MS), white (SPECT), or transparent (BSST) backgrounds. Although an overhead fluorescent lamp facilitated the reading of MS and SPECT reactions, the glare encountered on BSST cards necessitated viewing against a black background illuminated with an incandescent lamp. Positive reactions developed within 2 (MS), 4 (SPECT), or 10 min (BSST).

The SPECT system is based on two reagents (reagents 1 and 2) which together provide coverage for the A-E and G somatic serogroups and for the identification of the Vi antigen. Each reagent produces one of three colored reactions (red, blue, or green) as a result of its unique identification of a targeted serogroup or the Vi antigen. The BSST agglutination reactions of a goat polyclonal antibody (latex reagent 1) were rated visually on a scale of 1 to 4+. Positive reaction mixtures corresponding to > 2+ showed distinct clumps against a clear or cloudy background, whereas negative samples yielded a fine granular or cloudy background with no evidence of clumping. Reactions approaching a 2+ intensity were interpreted with some difficulty. The more clear-cut MS agglutination reactions were read according to the BSST rating scale. All latex kits suffered from the presence of suspended particulate matter during the direct assay of food enrichment cultures. Additionally, the green (TBG) and rusty-red (SC) coloration of enrichment cultures occasionally hampered the interpretation of SPECT reactions. All latex reactions in this study were read by a single analyst.

Standard cultural analysis.

Naturally contaminated foods (Table 2) were examined for *Salmonella* by a standard cultural procedure (*13*). Samples (100-g) were preenriched in nine volumes of NB for 18 h at 35°C (Fig. 1). Chocolate was similarly preenriched in 10% (w/v) reconstituted skim milk powder with added brilliant green dye to a final concentration of 0.002% (w/v). Replicate portions (1-ml) of each preenrichment culture were selectively enriched in nine volumes of tetrathionate brilliant green (TBG_{43}, TBG_{35}) and selenite cystine (SC_{35}) broths for 24 h at either 35 or 43°C. Each enrichment culture was then streaked on bismuth sulfite (BiS) and brilliant green sulfa (BGS) agar plates and incubated overnight at 35°C. Suspect colonies were screened biochemically on triple sugar iron and lysine iron agars, and confirmed serologically with polyvalent and single grouping antisera. All bacteriological media except LI (Oxoid, Columbia, MD) were obtained from Difco Laboratories (Detroit, MI).

RESULTS AND DISCUSSION

Of 190 *Salmonella* strains tested, the MS, BSST and SPECT identified 89.5, 81.6 and 66.3% of the test cultures, respectively (Table 1). It should be noted, however, that the sensitivity of the SPECT system increased to 92.7% when only serovars belonging to the SPECT-targeted serogroups were considered. One strain each of *Salmonella agona* and *Salmonella bardo* accounted for the SPECT false-negative results within somatic groups B and C, whereas five strains of *Salmonella thomasville*, two strains of *Salmonella drypool*, and one strain of *Salmonella halmstad* precipitated erroneous results within the test strains belonging to somatic group E. A similar weakness was

TABLE 2. Salmonella *detection in naturally contaminated foods.*[a]

Food	No. of samples		Serovars
	Tested	Positive (%)	
High moisture			
Poultry[b]	20	12	*S. hadar* (4); *S. heidelberg* (3); *S. thompson* (2); *S. branderup* (1); *S. kentucky* (1); *S. typhimurium* (1)
Pork	10	1	
Beef	2	0	*S. derby*
Lamb	1	0	
Snails	1	1	*S. miami*
Peanut butter	2	1	*S. tennessee*
Subtotal	36	15 (41.7)	
Low moisture			
Animal feed	10	3	*S. worthington* (2); *S. drypool* (1)
Egg noodles	4	1	*S. infantis*
Powdered drink	1	1	*S. tennessee*
Spices[c]	3	3	*S. glostrup* (1); *S. anatum* (1); *S. give* (1)
Milk chocolate	1	1	*S. nima*
Subtotal	19	9 (47.4)	
Total	55	24 (43.6)	

[a]Determined by the standard cultural procedure.
[b]Chicken carcasses (5), giblets (12), cut-up (1), minced chicken meat (1), and turkey/pork sausages (1).
[c]Black pepper (1), turmeric (1) and chili powder (1).

noted with the BSST reagents which failed to identify 12 (37.5%) of the 32 test strains within the somatic group E. Low BSST sensitivity (33.3%) for the 60 strains belonging to serogroups higher than somatic group E was also encountered. Although the MS kit showed the greatest sensitivity (89.5%), it also experienced difficulties in detecting members of uncommon somatic groups (Table 1). It is of equal interest that the flagellar antigen-directed MS antibodies detected all three nonmotile strains of *Salmonella gallinarum* but none of the three *Salmonella pullorum* strains tested in this study. Furthermore, 48 of the 190 test strains lacked phase 2 antigens; of these, 16 (33.3%) were not detected by MS antibodies and 11 (22.9%) strains gave weak agglutination reactions (data not shown). The lack of specificity of the MS (3.4%), SPECT (17.0%), and BSST (33.9%) kits with 59 nonsalmonellae cultures varied widely, with *Citrobacter freundii* and *Escherichia coli* precipitating most of the false-positive reactions (Table 1). Three different strains of *Aeromonas hydrophila* produced the single false-positive reaction obtained with each latex kit. The low specificity of BSST in this study contrasts with earlier reports of few aberrant reactions associated with the analysis of clinical samples (*10,12,17,18*). Although pure culture work failed to show reactivity of BSST antibodies with a limited number of strains of *Enterobacter cloacae* and *Hafnia alvei* (Table 1), it is noteworthy that such nonspecific reactions were encountered in subsequent studies with foods (Table 2). In the SPECT system, the anti-Vi reagent accounted for 7 (70%) of the 10 false-positive reactions attributed to this latex kit (Table 1).

Of 55 foods tested, 24 (43.6%) were found to contain *Salmonella* spp. by the standard cultural procedure (Table 2). No additional contaminated samples were detected by the latex kits. Although the sensitivity of the three latex kits increased with cumulative cultural steps, none of the kits identified all contaminated food samples when used in accordance to manufacturer's instructions (MS and SPECT), or when the latter instructions were arbitrarily applied to the clinical BSST system. Under such conditions, the MS, SPECT, and BSST assay of postenrichment cultures inoculated from TBG_{35} and SC_{35} identified 18 (75.0%), 19 (79.2%), and 21 (87.5%) contaminated samples, respectively (Table 3). The sensitivity of SPECT further increased to 95.7% if the single chocolate sample containing *Salmonella nima* (somatic group M which falls outside the range of SPECT coverage) was disregarded. The BSST kit stood alone in its ability to identify all contaminated high moisture foods, whereas none of the three latex kits showed unfailing sensitivity in the detection of contaminated low moisture foods. The ability of latex kits to detect salmonellae in enrichment cultures varied with the length of incubation periods. Direct latex assay of short (6 h) enrichment cultures yielded poor results with a maximum recovery rate of 50% with BSST (Table 3). Such lack of productivity likely stems from synergism between the threshold sensitivity of diagnostic reagents (i.e., 10^7 CFU/ml of SPECT, not stated for other kits), and the inability for short ($\leq$ 6 h) periods of selective enrichment to establish favorable *Salmonella* to nonsalmonellae ratios in enrichment cultures (*8*). It is likely that these factors also played in the poor performance of the diagnostic kits with preenrichment cultures (Table 3). Increasing the incubation period of selective enrichment from 6 to 24 h markedly enhanced the sensitivity of the three latex kits to levels ranging from 45.8 to 79.2%. A similar effect was previously noted in the BSST analysis of human stool samples enriched for 6 and 24 h in gram-negative (GN) broth (*18*). A more detailed examination of results by enrichment conditions is most revealing (Table 4). Although manufacturers of the MS and SPECT kits recommend that selected enrichment media such as TBG, SC, Rappaport-Vassiliadis, and cystine-mannitol be incubated at 35°C, it is important to underline the positive impact of elevated temperature (43°C) incubation of TBG in the present study. Specifically, postenrichment cultures inoculated from TBG_{43} facilitated the detection of 3 (MS), 1 (BSST), and 3 (SPECT) additional positive samples compared to that obtained with homologous TBG_{35} cultures (Table 4). Furthermore, plating of TBG_{43} (24 h) on BiS and BGS was the only enrichment-plating condition that identified all contaminated samples by the standard cultural procedure (data not shown). Such superiority of TBG_{43} undoubtedly emerges from the selective action of tetrathionate ($S_4O_6^=$) anions, and the high temperature-dependent repression of foodborne competitive microflora (*1,6,8*). The value of this analytical approach is well documented in the scientific literature and has been adopted in several standard methods (*13,16*).

The specificity of latex kits under combined enrichment conditions for foods ranked in the following decreasing order of merit: MS>SPECT>BSST (Table 4). This ranking corroborates earlier findings with pure cultures (Table 1). The number of false-positive reactions defined as the difference between the total (T) and culturally confirmed (C) agglutination reactions, substantially decreased as food analyses progressed towards the postenrichment step (Table 4) . More specifically, erroneous results were particularly prominent with preenrichment cultures where the rate of false-positive reactions (expressed as the percent ratio of erroneous positive reactions to the total number of food samples tested) varied from 10.9% (MS) and 12.7% (SPECT) to 21.8% (BSST). The few false-positive reactions with MS arose from preenrichment cultures. The SC_{35} enrichment cultures triggered varied responses from the three latex kits. In the BSST and SPECT systems, direct latex assay of SC_{35} (6 and 24 h) enrichments and derived postenrichment cultures generally engendered higher numbers of false-positive reactions compared to homologous TBG_{43} and TBG_{35} cultures. Notably, the high specificity of MS remained unshaken under SC_{35} enrichment conditions (Table 4). It is not inconceivable that the low selectivity of SC_{35} (*5,6*) allowed the proliferation of large populations of nonsalmonellae that readily reacted with BSST and SPECT antibodies. Interestingly, SC_{35} exerted a positive effect on BSST sensitivity as reflected in the identification of 11 (6 h) and 19 (24 h) contaminated samples, whereas direct BSST assay of homologous TBG_{43} and TBG_{35} cultures identified substantially fewer *Salmonella* positive samples. Although the underlying mechanism for the latter phenomenon remains unclear, it would be tempting to speculate that enrichment in SC_{35} more effectively stimulated the

TABLE 3. *Sensitivity of latex kits for detection of foodborne* Salmonella.

Kit	Food category[b]	No. of positive (confirmed) samples (%)[a]				
		Preenrichment	Selective enrichment		Postenrichment	
		18 h	6 h[c]	24 h[c]	Rec[d]	Max[c]
MS	H	1	0	8	12	12
	L	1	1	3	6	8
		2 (8.3)	1 (4.2)	11 (45.8)	18 (75.0)	20 (83.3)
BSST	H	6	8	14	15	15
	L	3	4	5	6	7
		9 (37.5)	12 (50.0)	19. (79.2)	21 (87.5)	22 (91.7)
SPECT	H	1	2	11	13	14
	L	0	0	4	6	8
		1 (4.2)	2 (8.3)	15 (62.5)	19 (79.2)	22 (91.7)

[a]Percent ratio of positive samples to total number of positive samples (24) by the standard method.
[b]High (H) and low (L) moisture foods.
[c]Based on combined results of TBG_{43}, TBG_{35} and SC_{35}.
[d]Based on combined results of TBG_{35} and SC_{35} as recommended by MS and SPECT manufacturers.

TABLE 4. *Specificity of latex kits in* Salmonella *food analyses*[a]

Kit	Food category[b]	No. of positive agglutination reactions																	
		Preenrichment 18 h		Selective enrichment 6 h						Selective enrichment 24 h						Postenrichment 6 h			
				TBG_{43}		TBG_{35}		SC_{35}		TBG_{43}		TBG_{35}		SC_{35}		TBG_{43}		TBG_{35}		SC_{35}	
		T[c]	C[c]	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
MS	H	4	1	0	0	1	1	0	0	3	3	2	2	7	7	11	11	10	10	6	6
	L	4	1	0	0	1	1	0	0	2	2	2	2	3	3	8	8	6	6	3	3
		8	2	0	0	2	2	0	0	5	5	4	4	10	10	19	19	16	16	9	9
BSST	H	8	3	6	5	6	5	9	7	11	10	9	8	18	14	17	15	17	14	17	14
	L	13	6	5	2	4	2	7	4	5	3	6	3	9	5	5	5	6	5	7	6
		21	9	11	7	10	7	16	11	16	13	15	11	27	19	22	20	23	19	24	20
SPECT	H	7	1	1	0	2	0	5	2	11	10	9	6	7	5	15	14	16	11	11	7
	L	1	0	0	0	0	0	1	0	4	4	2	2	2	1	6	6	7	6	4	3
		8	1	1	0	2	0	6	2	15	14	11	8	9	6	21	20	23	17	15	10

[a]Of 55 foods tested, 15/36 high moisture and 9/19 low moisture foods yielded salmonellae by the standard cultural method.
[b]High (H) and low (L) moisture foods.
[c]Total (T) and culturally confirmed (C) results.

production of BSST-specific *Salmonella* surface antigens. In the SPECT system, the latex reagent 2 precipitated all but two of the 31 false-positive (red-colored) Vi^+ reactions. Detailed consideration of these two non-Vi^+ related false-positive reactions showed that foodborne *C. freundii* interacted with the SPECT reagent 1 to yield a red-colored agglutination reaction product.

The present study of three *Salmonella* latex agglutination kits underlined the greater sensitivity and specificity of the MS system with pure cultures. Although the ability of kits to detect salmonellae under recommended (MS and SPECT) and arbitrarily applied (BSST) conditions of use was highest with BSST (Table 3), the efficacy of the latter system was overshadowed by the occurrence of substantial numbers of false-positive reactions. The limited serogroup coverage of the SPEC system and the occurrence of erroneous positive reactions at a rate comparable to BSST (Table 4) is of concern.

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