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Performance of the Microplate BacTrace™ ELISA Technique for Detection of Foodborne *Salmonella*

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ABSTRACT

The developmental BacTrace™ ELISA system which recognizes a common structural antigen (CSA-1) in the cell wall of target microorganisms was tested with pure cultures and naturally contaminated foods. The system readily detected all of the 104 *Salmonella* test strains but produced 38 (52.1%) false-positive reactions upon examination of 73 nonsalmonellae cultures. *Citrobacter freundii*, *Escherichia coli*, and *Proteus mirabilis* were primarily responsible for erroneous results. Of 119 foods tested, 37 (31.1%) were found to contain *Salmonella* by a standard cultural procedure. Parallel BacTrace™ testing of the nutrient broth (NB), tetrathionate brilliant green (TBG_{43}), and selenite cystine (SC_{35}) broth cultures arising from standard cultural analyses identified 24 (64.9%), 25 (67.6%), and 31 (83.8%) *Salmonella* contaminated foods, respectively. Maximum sensitivity of the test system (89.2%) could be attained through combination of ELISA results from both TBG_{43} and SC_{35}. False-positive reactions were particularly prominent with high moisture foods.

The widespread occurrence of *Salmonella* spp. in raw food materials of animal or botanical origin continuously challenges the efficacy of modern manufacturing processes in the production of safe food products. The massive daily throughput in the automated food industry and need for timely release of finished products underline the importance of rapid, cost-efficient, and reliable methods for the detection of bacterial pathogens in foods and food ingredients. Recent years have witnessed major advances in the development and validation of novel technologies for the detection of foodborne salmonellae. Such innovations have included colorimetric and fluorogenic enzyme-linked immunosorbent assay (ELISA) procedures (*5,7,11*), isotopic DNA-DNA and more recently chromogenic DNA-RNA probes (*9,12,16*), immunoimmobilization (*6*), and impedance (*8,15*) techniques.

The BacTrace™ microplate sandwich ELISA technique is based on the ability of an affinity purified polyclonal antibody to recognize a common structural antigen (CSA-1) located in the outer cell wall layer of *Salmonella* strains. The present study examines the sensitivity and specificity of the developmental BacTrace™ system for the detection of *Salmonella* spp. in pure cultures and in naturally contaminated foods.

MATERIALS AND METHODS

Test cultures

Pure cultures of *Salmonella* and nonsalmonellae (Table 1) were obtained from a laboratory collection of microorganisms maintained at room temperature on 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.

Sample preparation for BacTrace™ testing

A total of 104 *Salmonella* strains representing 20 somatic groups and 73 nonsalmonellae strains (12 genera, 19 species) were examined in this study. Stock bacterial cultures were subcultured twice in nutrient broth (NB) for 18 h at 35°C. A loopful of culture was then inoculated into 10 ml of NB and a 2-ml portion immediately withdrawn from the bacterial suspension (IN-SAMPLE). This sample was heated for 15 min in a boiling water bath and stored under refrigeration pending ELISA testing. The remaining portion (8 ml) of inoculated NB was incubated for 18 h at 35°C to an approximate cell concentration of 10^8/ml; a 2-ml portion of the culture (OUT-SAMPLE) was then withdrawn and boiled as previously described. The IN- and OUT-samples were equilibrated at room temperature and adjusted within the pH range of 5.0-7.0 prior to BacTrace™ assay. Negative media controls consisted of portions (2 ml) of uninoculated NB taken before (IN-SAMPLE) and after (OUT-SAMPLE) incubation for 18 h at 35°C.

For the analysis of foods by the standard cultural procedure (*13*), samples (100 g) were pre-enriched in nine volumes of nutrient broth (NB) for 18 h at 35°C. Replicate portions (1 ml) of each pre-enrichment culture were selectively enriched in tetrathionate brilliant green (TBG_{43}) and selenite cystine (SC_{35}) broths for 18 h at 43 and 35°C, respectively. 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 (TSI) and lysine iron (LI) agars and confirmed serologically with polyvalent and single grouping antisera.

The ability of the BacTrace™ system to provide greater method brevity was examined through application of the ELISA procedure to both pre-enrichment and selective enrichment cultures

of foods. During food analyses by the standard cultural procedure, portions (2 ml) from each NB, TBG_{43}, and SC_{35} broths were withdrawn before (IN-SAMPLE) and after (OUT-SAMPLE) incubation for 18 h at the appropriate temperature. IN- and OUT-samples were boiled and refrigerated as previously described. Negative media controls consisted of portions (2 ml) of uninoculated broths taken before (IN-SAMPLE) and after (OUT-SAMPLE) incubation.

ELISA procedure

In the microplate BacTrace™ (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD) procedure, all heat-treated IN- and OUT-samples were assayed according to manufacturer's instructions. Briefly, maxisorp immunoplates (Nunc, Denmark) were coated for 1 h at room temperature with capture antibody, and unoccupied protein binding sites were saturated with blocking solution. Duplicate 0.1 ml portions of IN- and OUT-samples were dispensed in appropriate microplate wells and incubated for 1 h at room temperature. The microplate was then emptied by rapid inversion and washed twice with Tween 20 in buffered saline to remove sample residues. After reaction of each well with a peroxidase-conjugated antibody for 1 h at room temperature, excess reagent was removed by repeated washings. Chromogenic ABTS (2,2'-azinobis (3-ethylbenzthiazoline sulfonate) substrate was then added to each well and allowed to react for 4 min. Microwells containing CSA-1 antigen yielded a green color which was read at a wavelength of 410 nm using a Minireader II (Dynatech Laboratories Inc.). An absorbance difference of ≥ 0.1 units between homologous IN- and OUT-samples was arbitrarily designated a positive reaction. Similar values have been used in earlier studies (*2,3*). The ELISA procedure usually required 4.5 h for completion, and a maximum of 22 pure cultures or food samples could be assayed on a single microplate.

TABLE 1. *BacTrace™ sensitivity and specificity with pure cultures.*

Microorganisms		Number of strains Tested[a]	Positive	Negative
Salmonella spp.				
	Somatic group B	14 (13)	14	0
	C	27 (26)	27	0
	D	9 (7)	9	0
	E	22 (16)	22	0
	F	2 (2)	2	0
	G	4 (4)	4	0
	H	3 (3)	3	0
	I	1 (1)	1	0
	others[b]	22 (13)	22	0
	subtotal	104 (85)	104	0
Other *Enterobacteriaceae*				
	Citrobacter freundii	17	17	0
	Enterobacter aerogenes	1	1	0
	Enterobacter agglomerans	4	2	2
	Enterobacter cloacae	1	1	0
	Enterobacter hafnia	1	0	1
	Escherichia coli	11	9	2
	Klebsiella pneumoniae	1	0	1
	Proteus mirabilis	10	7	3
	Proteus morganii	1	0	1
	Proteus rettgeri	1	0	1
	Proteus vulgaris	1	0	1
	Providencia spp.	1	0	1
	Shigella sonnei	1	0	1
	Shigella flexneri	1	0	1
	Yersinia enterocolitica	1	0	1
Nonrelated genera				
	Achromobacter xylosoxidans	1	0	1
	Aeromonas hydrophila	17	1	16
	Pseudomonas aeruginosa	1	0	1
	Staphylococcus aureus	1	0	1
	subtotal	73	38	35

[a]Numbers in brackets denote corresponding number of serovars.
[b]Relates to 12 additional somatic serogroups.

RESULTS AND DISCUSSION

The BacTrace™ CSA-1 antibody recognized the 104 strains (85 serovars) tested in the present study (Table 1). The intensity of *Salmonella*-dependent color reactions was determinate and generally corresponded to an $OD_{410} > 0.5$ above background (IN-SAMPLE) for the selected reaction time of 4 min. There was no evidence of a serogroup or serovar-dependent response to the ELISA reagents (data not shown). Interestingly, it has been reported that BacTrace™ sensitivity varies with *Salmonella* serovars and requires cell populations of $\geq 10^5$/ml for detection (*14*). Although BacTrace™ readily identified the 11 strains of *Salmonella arizonae* examined in the present study, two strains had yielded false-negative reactions in an earlier report (*17*). Thirty-eight (52.1%) of the nonsalmonellae strains tested produced false-positive results where *Citrobacter freundii, Escherichia coli,* and *Proteus mirabilis* accounted for most erroneous reactions. These findings are at variance with an earlier report on the unfailing specificity of the CSA-1 antibody with nonsalmonellae strains (*14*). The color intensity of false-positive reactions in the present work was substantially weaker than that encountered with *Salmonella* strains and showed an absorbance ranging from 0.2 to 0.6. Most strains of *C. freundii* and one strain of *Aeromonas hydrophila* were responsible for the higher absorbance values (data not shown).

The standard cultural procedure detected *Salmonella* in 25 (25%) of 100 high moisture and 12 (63.2%) of 19 low moisture foods (Table 2). Parallel BacTrace ™ analysis of standard broth cultures presumptively identified 82 (82%) high moisture and 15 (78.9%) low moisture foods of which only 33 (34.0%) could be confirmed by standard cultural results. Our findings correspond to an ELISA sensitivity of 89.2% and a false-positive rate of 53.8%. The greater rate of false-positive reactions with high moisture than with low moisture foods was not totally unexpected given the higher levels of background microflora in raw meats than in processed foods and the limited specificity of the BacTrace™ antibody (Table 1). Results of the present study contrast with a false-positive rate of 9.8% and absence of false-negative reactions reported in an earlier study of artificially contaminated beef and chicken slurries (*3*). A separate study had similarly encountered low rates of false-positive (5.5%) and false-negative (14%) reactions with naturally contaminated foods (*14*). Parallel BacTrace™ testing of the NB, TBG_{43}, and SC_{35} broth cultures arising from the standard cultural method identified 24 (64.9%), 25 (67.6%), and 31 (83.8%) *Salmonella* contaminated foods, respectively (Table 3). Our choice of a threshold absorbance difference of $OD_{410} \geq 0.1$ for positive reactions proved adequate in identifying culturally positive samples and produced rare indeterminate reactions. Association of 54 (45.4%) false-positive reactions with SC_{35} compared to 44 (37.0%) and 2 (1.7%) with NB and TBG_{43}, respectively, cautions against the use of SC_{35} enrichment cultures as the only test materials. Maximum sensitivity of the BacTrace™ system (89.2%) depended on a combination of ELISA results from both the TBG_{43} and SC_{35} cultures (Table 3). Comparable method sensitivity (86.0-87.5%) was previously reported following the examination of naturally and artificially contaminated foods and environmental samples (*2,14*). The intensity of color reactions with TBG_{43} and SC_{35} generally exceeded $OD_{410} > 1.00$ and contrasted with homologous NB color reactions which seldom attained $OD_{410} = 0.8$ (data not shown).

TABLE 2. Salmonella *detection in naturally contaminated foods.*

	Number of samples			
		Positive		
		Cultural	BacTrace[a]	
Food	Tested	Confirmed	Unconfirmed	Confirmed
High moisture				
Poultry				
carcasses/ cut-up	21	10	19	10
giblets	18	10	15	9
minced	2	0	2	0
burgers	1	0	0	0
sausages	1	0	1	0
Pork				
sausages	21	0	19	0
giblets	10	1	7	1
minced	8	1	7	1
Beef				
giblets	6	0	4	0
minced	4	0	4	0
Other foods				
froglegs	3	0	0	0
snails	1	1	0	0
lamb giblet	1	0	1	0
peanut butter	3	2	3	2
subtotal	100	25	82	23
Low moisture				
powdered drink	4	4	2	2
chocolate	1	0	0	0
black pepper	1	0	1	0
chili powder	1	1	1	1
egg noodles	1	1	1	1
animal feeds	11	6	10	6
subtotal	19	12	15	10
TOTAL	119	37	97	33

[a]Based on combined results from NB, TBG_{43}, and SC_{35} broth cultures.

TABLE 3. *BacTrace™ detection of* Salmonella *in pre-enrichment and enrichment cultures.*

Recovery patterns				Number of pattern reactions		
	BacTrace™					
Standard cultural	NB (24)[a]	TBG_{43} (25)	SC_{35} (31)	High moisture	Low moisture	Total
+	+	+	+	17	3	20
+	+	+	-	0	1	1
+	-	-	-	2	2	4
+	+	-	+	2	1	3
+	-	+	+	2	1	3
+	-	-	+	1	4	5
+	-	+	-	1	0	1
-	+	+	+	1	0	1
-	-	-	+	19	0	19
-	+	-	+	32	2	34
-	+	-	-	6	3	9
-	-	+	-	1	0	1
-	-	-	-	16	2	18
Total 37	68	27	85	100	19	119

[a]Total number of *Salmonella* contaminated samples confirmed by the standard method.

Examination of the productivity of different enrichment-plating conditions underlined the greater sensitivity of the TBG_{43} + BiS combination for both high moisture and low moisture foods (Table 4). Plating of both TBG_{43} and SC_{35} on BiS provided the only cultural conditions that identified all positive samples. Selective enrichment at an elevated temperatures (41-43°C) and high productivity of the saccharide-independent BiS plating medium undoubtedly played a determinant role in the repression of competitive microflora and facilitated recovery of salmonellae (*1,2,4,5*).

The present study demonstrated that the sensitivity of the BacTrace™ system compared favorably with other ELISA kits for the detection of foodborne *Salmonella* (*7,10,11*). Our results indicate that use of combined TBG_{43} and SC_{35} results is necessary to maximize BacTrace™ sensitivity. Reliability of the assay system with pooled TBG_{43} and SC_{35} cultures was not examined in our study and would need to be ascertained experimentally. The number of false-positive reactions encountered with high moisture foods places certain reservations on the overall performance of the BacTrace™ system and underscores a need to improve the specificity of the CSA-1 antibody.

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TABLE 4. *Productivity of* Salmonella *enrichment-plating conditions.*

		Salmonella positive samples							
		TBG_{43}		SC_{35}		TBG_{43} + SC_{35}		TBG_{43}	SC_{35}
Food	Number positive samples[a]	BiS	BGS	BiS	BGS	BiS	BGS	BiS + BGS	
High moisture	25	24	23	23	16	25	23	24	23
Low moisture	12	11	9	10	10	12	12	11	10
Totals	37	35	32	33	26	37	35	35	33
Percent	100	95	86	89	70	100	95	95	89

[a]Based on combined cultural results.

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