MICROBIOLOGICAL METHODS

Paradigm Diagnostics Salmonella Indicator Broth (PDX-SIB) for Detection of *Salmonella* on Selected Environmental Surfaces

Performance Tested Method[SM] 071102

Abstract

The Paradigm Diagnostics Salmonella Indicator Broth (PDX-SIB) is intended as a single-step selective enrichment indicator broth to be used as a simple screening test for the presence of *Salmonella* spp. in environmental samples. This method permits the end user to avoid multistep sample processing to identify presumptively positive samples, as exemplified by standard U.S. reference methods. PDX-SIB permits the outgrowth of *Salmonella* while inhibiting the growth of competitive Gram-negative and -positive microflora. Growth of *Salmonella*-positive cultures results in a visual color change of the medium from purple to yellow when the sample is grown at 37 ± 1°C. Performance of PDX-SIB has been evaluated in five different categories: inclusivity-exclusivity, methods comparison, ruggedness, lot-to-lot variability, and shelf stability. The inclusivity panel included 100 different *Salmonella* serovars, 98 of which were SIB-positive during the 30 to 48 h incubation period. The exclusivity panel included 33 different non-*Salmonella* microorganisms, 31 of which were SIB-negative during the incubation period. Methods comparison studies included four different surfaces: *S.* Newport on plastic, *S.* Anatum on sealed concrete, *S.* Abaetetuba on ceramic tile, and *S.* Typhimurium in the presence of 1 log excess of *Citrobacter freundii.* Results of the methods comparison studies demonstrated no statistical difference between the SIB method and the U.S. Food and Drug Administration-*Bacteriological Analytical Manual* reference method, as measured by the Mantel-Haenszel Chi-square test. Ruggedness studies demonstrated little variation in test results when SIB incubation temperatures were varied over a 34–40°C range. Lot-to-lot consistency results suggest no detectable differences in manufactured goods using two reference *Salmonella* serovars and one non-*Salmonella* microorganism.

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The method was independently tested, evaluated, and certified by the AOAC Research Institute as a *Performance Tested Method*[SM]. *See* http://www.aoac.org/testkits/steps.html for information on certification.

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Scope of Method

(**a**) *Target organisms*.—*Salmonella.*

(**b**) *Matrixes*.—Plastic, sealed concrete, ceramic tile, and stainless steel.

(**c**) *Summary of validated performance claims*.—The overall sensitivity relative to the reference method across all four surfaces was >100% (it is possible bacterial growth occurred during the study period). There were no significant differences between the Paradigm Diagnostics Salmonella Indicator Broth (PDX-SIB) method and the reference method on any of the surfaces tested.

Definitions

(**a**) *Relative sensitivity*.—Defined as the number of samples testing positive by the SIB method divided by the number of samples testing positive by the reference culture procedure.

(**b**) *Statistical data analysis*.—The Mantel-Haenszel Chi-square formula for unmatched test portions was used for the statistical analysis (1). A Chi-square value <3.84 indicates that the proportions positive for the alternative and the reference methods are not statistically different at the 5% level of significance. This criterion must be satisfied for each level of each surface type. However, a significant difference between the proportions positive for the two methods is acceptable provided that recovery by the alternative method is superior to that by the reference method.

Principle

The principle of PDX-SIB utilizes two operating conditions: selective enrichment of the *Salmonella* population from the background microflora; and the simultaneous metabolism of a very specific *Salmonella* substrate. PDX-SIB is a balanced blend of proprietary selective agents highly restrictive to non-*Salmonella* bacteria and combining a highly specific metabolic substrate for *Salmonella.* As the selected population grows out, the media becomes acidified and an incorporated pH indicator detects the pH change by a color shift from purple to yellow.

General Information

Foodborne salmonellosis remains a major public health problem. Although the genus *Salmonella* has more than 2300 serovars, only a relatively restricted number, belonging mainly to the species *Salmonella enterica* subsp. *enterica,* are responsible for most human infections. All these serovars are primarily pathogenic for animals, so the most common sources of *Salmonella* infections for humans are contaminated foods such as eggs, poultry, produce, meat, and meat products. In a survey conducted in the United States, Mead et al. (2) estimated that in 1999 nontyphoidal salmonellosis of foodborne origin caused approximately 15 600 hospitalizations and 550 deaths. *Salmonella* is, thus, in second place for the number of annual illness cases, with an estimated 1 340 000 cases, compared to nearly 2 000 000 for *Campylobacter*. Eggs and poultry meat have been recognized as the major vehicles of human infections because of epizootics in fowl.

Early detection of the pathogens is vital for food safety assurance. Because conventional methods for *Salmonella* detection in foods are long and time-consuming, early detection is needed if foodborne illnesses caused by *Salmonella* are to be reduced. Routine analytical methods involve selective enrichment and plating on selective differential agar followed by biochemical and immunochemical characterization (3). It is estimated that millions of *Salmonella* analyses are run routinely in the United States each year. Simultaneous recovery and detection of *Salmonella* using a single testing method will reduce time and labor and media costs. Streamlining the procedure and reducing labor and test costs should permit more frequent monitoring for *Salmonella*, thereby reducing contamination hazard.

Material and Methods

Test Kit Information

(**a**) *Kit name*.—PDX-SIB.

(**b**) *Cat. Nos*.—260003-25, 260009-50, 260004-100.

(**c**) *Ordering information*.—*Inside the United States*.—Paradigm Diagnostics, Inc., 800 Transfer Rd, Suite 12, St. Paul, MN 55114, Tel: 651-226-0381, Fax: 651-405-0490, website: www.pdx-inc.com.

(**d**) *Kit components*.—One 15 mL screw-cap vial containing PDX-SIB.

Additional Supplies Recommended

Enviroswabs are available through 3M Co. or Paradigm Diagnostics, Inc., or equivalent.

Alternatives include Whirl-Pak™ bags with premoistened carcass sponges or equivalent.

Apparatus

Temperature-thermostatted incubator (preferred) or heating block (32 to 40°C).

Standard Reference Materials

Reference strains were obtained through the following sources: Microbiologics, Inc., St. Cloud, MN; National Collection of Type Cultures (NCTC) Health Protection Agency Culture Collection, Porton Down, UK; Francisco Diez-Gonzalez, Department of Food Science, University of Minnesota, St. Paul, MN; and the American Type Culture Collection (ATCC), Bethesda, MD. Serogroups were identified through reference to the handbook of *Salmonella* antigenic structures (4).

Safety Precautions

The PDX-SIB test should be performed by personnel with appropriate microbiology laboratory training. Material Safety Data Sheets are available upon request. All materials used should be handled and disposed of as potentially infectious material. Autoclaving is the preferred method of disposal. If autoclaving is not available, disinfectant solutions should be used; the disinfection protocol should be validated to inactivate the microorganisms. Most *Salmonella* are human pathogens. When handling samples and enriched broths that possibly contain *Salmonella*, care should be taken to contain the samples and the enriched samples (presumptive positive tubes). Immunocompromised individuals are particularly sensitive to infection by *Salmonella* spp. and should not be allowed in the vicinity of the testing.

General Preparation

Pure cultures were obtained from NCTC; Microbiologics, Inc.; University of Pennsylvania School of Veterinary Medicine, Salmonella Reference Center; and the microbiology laboratories of Francisco Diez-Gonzalez and Joellen Feirtag at the University of Minnesota. Original pure cultures were grown overnight in sterile tryptic soy broth (TSB) or brain heart

Table 1. Results of inclusivity test for PDX-SIB

Serovar	Source	Origin	SIB medium color	Presumptive result	Serogroup
S. Adelaide	U of MN 94679420	Meat meal	Yellow	+	O
S. Agona	U of MN inv 95650951	Soybean meal	Yellow	+	B
S. Albany	U of MN 2009595	Frozen fish paste	Yellow	+	C3
S. Anatum	U of MN 95645854	Chicken feed	Yellow	+	E1
S. Bovismorbificans	U of MN 3064124	Vietnam	Yellow	+	C2
S. Carrau	U of MN 2003413	Frozen shrimp	Yellow	+	H
S. Cerro	U of MN 94713965	Poultry feed	Yellow	+	K
S. Cubana	U of MN 94679421	Swine feed	Blue	–	G2
S. Chester	U of MN 3063650	Frozen tilapia fish	Yellow	+	B
S. Emek	U of MN 3063892	Frozen catfish	Yellow	+	C3
S. Enteritidis	U of MN 95657613	Ice cream	Yellow	+	D1
S. Give	U of MN 1829352	Lobster tail	Yellow	+	E1
S. Gloucester	U of MN 1676771	Sesame seeds	Yellow	+	B
S. Hvittingfoss	U of MN 200373	Frozen frog legs	Yellow	+	I
S. Infantis	U of MN 2015422	Frozen lobster tail	Yellow	+	C1
S. Javiana	U of MN 1842147	Frozen shrimp	Yellow	+	D1
S. Kentucky	U of MN 95–690–012	Cottonseed meal	Yellow	+	C3
S. Lille	U of MN 95–713–959	Chicken feed	Yellow	+	C1
S. Mbandaka	U of MN 95690014	Soybean meal	Yellow	+	C1
S. Meleagridis	U of MN 1949345	Frozen shrimp	Yellow	+	E1
S. Montevideo	U of MN 95573493	Raw eggs	Yellow	+	C1
S. Muenchen	U of MN 1842204	Frozen shrimp	Yellow	+	C2
S. Newbrunswick	U of MN 1842304	Frozen shrimp	Yellow	+	E1
S. Nashua	U of MN 2006036	Poultry feed	Yellow	+	M
S. Newport	U of MN 2006038	Frozen lobster tail	Yellow	+	C2
S. Penilla	U of MN 1949289	Frozen shrimp	Yellow	+	M
S. Poona	U of MN 1103174	White pepper	Yellow	+	G1
S. Sterrenbos	U of MN 1842082	Frozen shrimp	Yellow	+	C3
S. Thompson	U of MN 95657618	Ice cream	Yellow	+	C1
S. Weltevreden	U of MN 1950358	Dried ling shrimp	Yellow	+	E1
S. Typhimurium	U of MN 3019907	Salted dune egg	Yellow	+	B
S. Worthington	U of MN 95–713–958	Chicken feed	Yellow	+	G2
S. Kumasi	U of MN 1929854	Frozen crab meat	Yellow	+	N
S. Rubislaw	U of MN 2004976	Frozen shrimp	Yellow	+	F
S. Goodwood	U of MN	Feces	Yellow	+	E4
S. Senftenberg	U of MN	Sewage	Yellow	+	E4
S. Ohio	U of MN	Animal feed	Yellow	+	C1
S. Limete	U of MN		Yellow	+	B
S. Tennessee	U of MN	Soybean meal	Yellow	+	C1
S. Newington	U of MN	Wild poultry	Yellow	+	B
S. Aberdeen	NCTC 5791	Infantile diarrhea	Yellow	+	F
S. Aequatoria	NCTC 7891	African zoonosis	Yellow	+	C1
S. Alabama	NCTC 9868	Human feces	Yellow	+	B
S. Altendorf	NCTC 10546		Yellow	+	B
S. Austin	NCTC 8447		Yellow	+	C1
S. Ball	NCTC 9870		Yellow	+	B
S. Berkeley	NCTC 8260	Diseased turkey	Yellow	+	U
S. Brookfield	NCTC 10946		Yellow	+	O66
S. California	NCTC 6018	Animal feed	Yellow	+	B

Table 1. (*continued*)

Serovar	Source	Origin	SIB medium color	Presumptive result	Serogroup
S. Canastel	NCTC 6948	Animal feed	Yellow	+	D1
S. Carmel	NCTC 9872	Infantile diarrhea	Yellow	+	O17
S. Champaign	NCTC 6851	Hen liver	Yellow	+	Q
S. Chicago	NCTC 9873		Yellow	+	M
S. Colombo	NCTC 9922	Sheep	Yellow	+	P
S. Ealing	NCTC 11949	Dried baby milk	Yellow	+	O
S. Dahlem	NCTC 9949	Cattle	Yellow	+	Y
S. Gallinarum	NCTC 10532	Poultry	Blue	–	D1
S. Houten	NCTC 10401	Reptile	Yellow	+	O43
S. Kottbus	NCTC 5753	Feces	Yellow	+	C2
S. Illinois	NCTC 8498	Poults	Yellow	+	E3
S. Lexington	NCTC 6244	Soybean	Yellow	+	E1
S. Manchester	NCTC 7372		Yellow	+	C2
S. Minnesota	NCTC 5800	Swine	Yellow	+	L
S. Mississippi	NCTC 6487	Feces	Yellow	+	G2
S. Napoli	NCTC 6853	Food handlers	Yellow	+	D1
S. Pensacola	NCTC 6946		Yellow	+	D1
S. Pretoria	NCTC 6234	Meat	Yellow	+	F
S. Shanghai	NCTC 9791		Yellow	+	I
S. Sunsvall	NCTC 9787	Dried egg	Yellow	+	H
S. Waycross	NCTC 7401	Urine	Yellow	+	S
S. Alachua	U Penn STS 6	Swine	Yellow	+	O
S. Choleraesuis	ATCC 10708	Fish	Yellow	+	C
S. Arkansas	U Penn STS 11		Yellow	+	B
S. Blockley	U Penn STS 15	Environment	Yellow	+	C2
S. Brandenburg	U Penn STS 18	Swine	Yellow	+	B
S. Derby	U Penn STS 22	Polluted water	Yellow	+	B
S. Dublin	U Penn STS 27	Cattle	Yellow	+	D1
S. Hadar	U Penn STS 45	Turkey	Yellow	+	C2
S. Heidelberg	U Penn STS 48	Poultry	Yellow	+	B
S. London	U Penn STS 64	Polluted water	Yellow	+	E1
S. Manhattan	U Penn STS 65	Avian	Yellow	+	C2
S. Oranienburg	U Penn STS 83	Egg	Yellow	+	C1
S. Panama	U Penn STS 86	Infantile diarrhea	Yellow	+	D1
S. Paratyphis	ATCC 13314	Sewage	Yellow	+	A
S. Saint Paul	U of MN	Milk powder	Yellow	+	B
S. Schwarzengrund	U Penn STS 95	Chicken	Yellow	+	B
S. Stanley	U Penn STS100	Reptile	Yellow	+	B
S. Urbana	U Penn STS110	Reptile	Yellow	+	N
S. Johannesburg	U Penn STS 56	Meat meal	Yellow	+	R
S. Thomasville	U Penn STS103	Poultry meal	Yellow	+	E3
S. Virchow	U Penn STS 112	Basil	Yellow	+	C1
S. Abaetetuba	ATCC 35640	Fresh water	Yellow	+	F
S. Choleraesuis var. Kunzendorf	ATCC 12011	Swine	Yellow	+	B
S. Vallore	ATCC 15611		Yellow	+	B
S. Paratyphis	U of MN 2014696	Frozen frog legs	Yellow	+	B
S. Tallahassee	ATCC 12002		Yellow	+	C3
S. Salford	U of MN 2009532	Oregano turkey	Yellow	+	I

Table 1. (*continued*)

Serovar	Source	Origin	SIB medium color	Presumptive result	Serogroup
S. Birmingham	U of MN DI95764802	Alfalfa seed	Yellow	+	E1
S. Brunei	U of MN 1680318	Frozen shrimp	Yellow	+	C3
S. Ikeja	U of MN 3019543	Frozen shrimp	Yellow	+	E1
S. Cubana	U Penn	Swine	Yellow	+	G2

infusion (BHI) broth at 32–37°C. In order to have reproducible cultures, 50% glycerol stock solutions of cultures were prepared and stored in a freezer. Fifty percent glycerol stock cultures were prepared by diluting 500 µL of pure culture grown overnight with 500 µL sterile glycerol. Then 200 µL portions were transferred into sterile 2 mL centrifuge tubes, capped, and kept in a freezer at –15°C until the day before use. A loop-full of freezer-stored stock cultures was then inoculated into 5 mL sterile TSB, and the same loop was streaked onto tryptic soy agar (TSA). The plates were incubated overnight at 37 ± 1°C. TSA plates were checked for visible contamination, based on colony morphology. If the TSA plates suggested no visible contamination, logarithmic level dilutions were made into sterile peptone solutions (0.1% peptone water). Aliquots containing 100 µL of 6 and 7 log dilutions from overnight cultures were plated onto TSA for estimating the cell concentration (CFU/mL) and checked for contamination.

Sample Preparation

Sampling for AOAC Research Institute (RI) guideline studies.—Dilutions of pure cultures mentioned above were used for all AOAC-RI recommended studies: inclusivity-exclusivity, method comparison, ruggedness, lot-to-lot variability, and shelf stability studies.

Analysis

Take an environmental sample following U.S. Food and Drug Administration-*Bacteriological Analytical Manual* (FDA-BAM) recommendations for sampling (3). Return the sampling device to the original sterile container. Aseptically add one unit (15 mL) of PDX-SIB media to the sterile container, fully submerging the applicator tip. Incubate in upright position for 30–48 h at 37 ± 1°C. Check the color of the media. Sample is called presumptive positive for *Salmonella* if color of the media changed from purple to yellow.

Interpretation and Test Result Report

Bright yellow medium and sponge at the end of 30–48 h of incubation indicates presumptive presence of *Salmonella*. In order to confirm the negatives, all purple/pale colorless (presumptive negative) tubes must be incubated for a total of 48 h. It is recommended that at least one negative control be run in each set of analysis. A negative control is an unused sampling device containing one unit of PDX-SIB, incubated alongside the samples.

Internal Validation Studies

Inclusivity-Exclusivity

Serial dilutions from overnight grown pure cultures of 100 different *Salmonella* and 33 different non-*Salmonella* cultures were made into sterile 0.1% peptone water. A 100 µL volume from log –8 dilution (about 1.0E + 3 CFU/mL) was plated onto TSA plates for estimating the number of cells tested. For the inclusivity studies, 0.1 mL (estimated cell concentration ranging from 10 to 100 CFU of *Salmonella*) of a dilution aliquot was aseptically transferred onto the top of the sampling sponge. For the exclusivity studies, 0.2 mL (estimated cell concentration >1 000 000 CFU of non-*Salmonella*) of a dilution aliquot was aseptically transferred onto the top of the sampling sponge. The sampling device was then placed back into the sterile tube and submerged in 15 mL of PDX-SIB media. The results are given in Tables 1 and 2.

Method Comparison Studies

Method comparison studies were done for four different *Salmonella* species paired with four different common food environmental surfaces: *S.* Abaetetuba on ceramic tiles, *S.* Anatum on sealed concrete, *S.* Newport on plastic surface, and *S.* Typhimurium in the presence of 1 log excess *C. freundii* on stainless steel.

(**a**) *FDA-BAM method.*—The FDA-BAM method calls for a multistep procedure as described. Following the 2 h holding period after sampling, Enviroswabs were transferred to sterile stomacher bags and enriched with 225 mL lactose broth. The samples were allowed to stand for 60 ± 5 min at room temperature, then incubated for 24 ± 2 h at 35 ± 1°C. No pH adjustment was necessary prior to incubation. After incubation, 0.1 mL of primary enrichment for each sample was transferred to 10 mL Rappaport-Vasilliadis (RV) medium, and 1.0 mL was transferred to 10 mL tetrathionate (TT) broth. The RV broth was incubated at 42 ± 1°C for 24 ± 2 h and the TT broth at 35 ± 1°C for 24 ± 2 h. Following incubation, a loopful (10 µL) of each secondary enrichment was streaked to bismuth sulfate (BS), xylose lysine desoxycholate (XLD), and Hektoen enteric (HE) selective agars and incubated at 35 ± 1°C for 24 ± 2 h. The BS plates that were negative at 24 h were then reincubated for an additional 24 h at 35 ± 1°C. A suspect colony from each selective agar was picked and stabbed to triple sugar iron (TSI) agar and lysine iron agar (LIA) plates, and streaked to a TSA plate. The TSI, LIA, and TSA plates were incubated at 35 ± 1°C for 24 ± 2 h. Growth from each TSA plate was used to conduct the polyvalent somatic O serological Microgen® Salmonella Latex test (Microgen Bioproducts Ltd, Surrey, UK), and biochemical tests for confirmation. Final confirmations were

Table 2. Results of exclusivity test for PDX-SIB

Species	Source	Origin	SIB result
Klebsiella pneumoniae	NCTC 9633	Sputum	–
Proteus mirabilis	ATCC 12453	GI tract	–
Citrobacter freundii	NCTC 9750	Soil	–
Escherichia coli	ATCC 13706	GI tract	–
Escherichia coli	ATCC 14948	GI tract	–
Hafnia alvei	ATCC 700025	Brewery fermentation samples	–
Serratia liquefaciens	ATCC 27592		–
Morganella morganii subsp. *morganii*	ATCC 25829		–
Pseudomonas aeruginosa	ATCC 10145		–
Providencia rettgeri	ATCC 9250		–
Enterobacter amnigenus	ATCC 51816		–
Enterobacter aerogenes	ATCC 13048		–
Shigella sonnei	ATCC 25931		–
Shigella flexneri	ATCC 9199		–
Staphylococcus epidermidis	ATCC 14990		–
Staphylococcus aureus	ATCC 700699		–
Serratia marcescens	ATCC 13880	Polenta	–
Enterobacter cloacae subsp. *cloacae*	ATCC 23355		–
Enterobacter gergoviae	ATCC 33028		–
Klebsiella oxytoca	ATCC 13182		–
Providencia wickerhamii	ATCC 16529		–
Shigella boydii	ATCC 9207		–
Staphylococcus aureus	NCTC 12973		–
Yersinia enterocolitica subsp. *enterocolitica*	ATCC 23715		–
Yersinia ruckerii	ATCC 29473		–
Citrobacter freundii	ATCC 8090		+
Citrobacter braakii	ATCC 43162		–
Citrobacter koseri	ATCC 27156		+
Escherichia coli	NCIMB 11943		–
Escherichia coli	NCTC 10538		–
Listeria monocytogenes	ATCC 13932		–
Listeria innocua	ATCC 33090		–
Pasteurella multocida subsp. *multocida*	ATCC 12945		–
Providencia stuartii	ATCC 33672		–
Edwardsiella tarda	ATCC 15947		–

conducted with Gram-negative (GN-ID) biochemical panels (Microgen).

(**b**) *PDX-SIB method*.—Following the 2 h holding period after sampling, excess Dey-Engley (DE) neutralizing broth from the Enviroswab tubes was removed and 15 mL *Salmonella* indicator broth was added to the Enviroswab tube. The samples were then incubated at 37 ± 1°C for 24–48 h. Each sample was then examined for presumptive positive results (broth color change from blue to yellow). Presumptive positives at 24 h were streaked to the reference agars for the specified reference method and reincubated for an additional 24 h. Positive and negative samples, yellow and purple respectively, were streaked onto XLD, HE, and BS and followed through to confirmation as described for the FDA-BAM method.

(*1*) *S. Newport on plastic surface*.—Serial logarithmic dilutions of overnight culture of *S.* Newport in BHI were made into sterile BHI. A 100 µL volume of log –7 and –8 dilutions was plated onto TSA for estimating the number of cells loaded onto 4 × 4 in. zones on plastic. Polypropylene plastic cutting boards were bought from a local department store. The 4 × 4 in. zones were marked on the surface of the cutting boards, which were wrapped in aluminum foil and autoclaved for 15 min at 120°C. The plastic cutting boards were kept wrapped until the time of inoculation. Surfaces were inoculated at 0.5 mL/4 × 4 in. surface from the log –7 (high) and –8 (low) dilutions, corresponding approximately to 2.18 and 1.18 log CFU of *S.* Newport consecutively, 20 replicates for each level/method. Inoculations on surfaces were spread within the corresponding 4 × 4 in. zone with the help of a disposable sterile spreader. After that, surfaces were left to dry for a minimum of 18 h; they were then removed by swabbing using Tecra Enviroswabs saturated with 10 mL DE broth. Both the test method (PDX-SIB) and the reference method (FDA-BAM) were done in 20 replicates/level (high and low) of inoculations. In order to confirm the presence of *Salmonella*; all test samples were streaked onto HE, XLD, and BS agar after 48 h at 37 ± 1°C. Dark green colonies grown on HE were selected and processed as described for GN-ID analysis and polyvalent somatic O serological tests. Five uninoculated samples were assayed. Data for *S.* Newport on plastic are given in Table 3.

Table 2 shows that PDX-SIB was comparable to the reference method. The reference method gave two more positives at the low level than the test method. The Chi-square values indicated that the test method and the reference method were not significantly different at the low level.

(*2*) *S. Anatum on sealed concrete*.—*S.* Anatum stock culture was grown overnight in BHI. Serial logarithmic dilutions of *S.* Anatum were made into sterile BHI. A 100 µL volume of log –7 and –8 dilutions was plated onto TSA plates for estimating the number of cells loaded onto 4 × 4 in. zones on sealed concrete blocks. Concrete blocks (15.5 L × 7.5 W × 3.5 in. D) were purchased from a local Home Depot. Blocks were sealed with a solvent-based concrete sealer (BW Crete Seal 25 LV, St. Paul, MN). After a minimum of two coats of sealant were placed on the blocks, they were dried in a chemical hood until all the solvent had evaporated (minimum of 24 h). Zones of 4 × 4 in. were marked on the sealed side using a permanent marker. Before inoculations, sealed concrete blocks were sprayed with ethanol and allowed to air-dry for not more than an hour. The inoculation of sealed concrete surfaces were done at 0.5 mL/surface from the log –7 *S.* Anatum (high) and log –8 *S.* Anatum (low) dilutions, corresponding approximately to 2.22 and 1.25 log CFU, respectively. Inoculations on surfaces were spread within the corresponding 4 × 4 in. zone. Surfaces were left to dry for at least 18 h at room temperature; they were then removed by swabbing with Tecra Enviroswabs. Both the test method (PDX-SIB) and the reference method (FDA-BAM) were done in 20 replicates/level (high and low) of inoculations. Five uninoculated samples were assayed.

Table 3 shows that the reference method identified a single

Table 3. Summary of method comparison studies of SIB at 48 h incubation

			PDX-SIB		FDA-BAM		
Matrix	Strain	N^a	Presumptive positive	Confirmed positive	Positive	Chi-square[b]	Relative sensitivity, %[c]
Plastic	*S.* Newport	5	0	0	0	—	—
		20 Low level	11	11	13	0.406	84.6
		20 High level	20	20	20	0	100
Sealed concrete	*S.* Anatum	5	0	0	0	—	—
		20 Low level	7	7	8	0.104	87.5
		20 High level	20	20	20	0	100
Ceramic tile	*S.* Abaetetuba	5	0	0	0	—	—
		20 Low level	10	10	7	0.898	142.9
		20 High level	20	20	20	0	100
Stainless steel[d]	*S.* Typhimurium: 10X *C. freundii*	5	0	0	0	—	—
		20 Low level	0	0	2	2.05	0
		20 High level	5	5	3	0.609	166.7

[a] *N* = Number of test portions.

[b] Chi-square = Mantel–Haenszel: $\chi^2 = (n - 1)(ad - bc)^2/[(a + b)(a + c)(b + d)(c + d)]$, where *n* = total number of samples tested by the two methods, a = number of samples positive by the test method, b = number of samples negative by the test method, c = number of samples positive by the reference method, and d = number of samples negative by the reference method.

[c] Relative sensitivity = a/c, where a = number of samples confirmed positive by the test method, and c = number of samples positive by the reference method.

[d] Trial performed at the independent laboratory.

sample more than the test method at the low level. Accordingly, Chi-square values suggested that there was no significant difference between the test method and the reference method at both levels tested.

(*3*) *S. Abaetetuba on ceramic tile*.—Serial logarithmic dilutions of overnight culture of *S.* Abaetetuba in BHI were made into sterile BHI. Aliquots containing 100 µL of log –8 and –9 dilutions were plated onto TSA to estimate the number of cells loaded onto 4 × 4 in. ceramic tiles, which were bought at a local Home Depot. The tiles were wrapped in aluminum foil, autoclaved for 15 min at 121°C, and kept wrapped at room temperature until inoculation.

Surfaces were inoculated at 0.50 mL/4 × 4 in. surface from the log –8 (high) and log –9 (low) dilutions, corresponding approximately to 1.67 and 0.60 log CFU, respectively, with 20 replicates for each level/method. Inoculations on surfaces were spread within the corresponding 4 × 4 in. zone with the help of sterile spreaders. Surfaces were left to dry for at least 18 h at room temperature, then removed by swabbing using the Tecra Enviroswabs. Both the test method (PDX-SIB) and the reference method (FDA-BAM) were done in 20 replicates/level (high and low) of inoculations. Five uninoculated samples were assayed.

In order to confirm the presence/absence of *Salmonella* in all test samples, all tubes of PDX-SIB were streaked onto HE, XLD, and BS plates after 48 h of incubation. Dark green colonies grown on HE were processed as described for immunoassay and GN-ID analysis for confirmation. Data for *S.* Abaetetuba on tile are given in Table 3.

Table 3 shows that PDX-SIB was slightly more sensitive than the reference method. The reference method resulted in fewer positives (seven versus 10) than the test method at the low level. The Chi-square value also showed that the two methods were not significantly different from each other at the low-level testing.

(**c**) *Independent laboratory surface comparison study of S. Typhimurium and C. freundii on stainless steel*.—For the analysis of stainless steel surfaces, a total of 45 samples for both the PDX-SIB and FDA-BAM were analyzed for method comparison. Within each sample set, there were 20 low-level, 20 high-level, and five uninoculated samples. The target levels of *S.* Typhimurium ATCC 14028 used for challenging the stainless steel surfaces were as follows: 1–50 CFU/4 × 4 in. (100 cm^2) surface area for low-level samples; 50–100 CFU/4 × 4 in. (100 cm^2) surface area for high-level samples; and 0 CFU/4 × 4 in. (100 cm^2) surface area for uninoculated control samples. Additionally, the surfaces were inoculated with *C. freundii* ATCC 8090 at 10 times the level of *S.* Typhimurium to simulate performance of the target organism in the presence of a competing microflora. The inocula were prepared in BHI broth incubated for 24 ± 2 h at 37 ± 1°C. After incubation, the broth inocula were serial diluted with BHI until the target inoculum level was reached. The stainless steel surfaces were inoculated with 0.25 mL from both the *S.* Typhimurium and *C. freundii* inocula and allowed to dry at ambient temperature for 16–24 h.

Results obtained from the stainless steel environmental surfaces assayed by the PDX-SIB method were comparable to those analyzed by the FDA-BAM reference method for the detection of *Salmonella*. A Mantel-Haenszel Chi-square analysis for unmatched test portions of the PDX-SIB method and reference methods produced a value of 2.05 for the low-level test portions and 0.61 for the high-level portions. The values

Table 4. Temperature variability

	24 h			48 h		
Strain	34°C	37°C	40°C	34°C	37°C	40°C
			S. Abaetetuba			
A	+	+	+	+	+	+
B	+	+	+	+	+	+
C	+	+	+	+	+	+
D	+	+	+	+	+	+
E	+	+	+	+	+	+
			S. Anatum			
A	+	+	–	+	+	+
B	+	+	–	+	+	+
C	+	+	–	+	+	+
D	+	+	–	+	+	+
E	+	+	–	+	+	+
			E. coli			
A	–	–	–	–	–	–
B	–	–	–	–	–	–
C	–	–	–	–	–	–
D	–	–	–	–	–	–
E	–	–	–	–	–	–

obtained for the matrix indicate that there was no statistically significant difference between the number of confirmed positive results obtained by the two methods being compared at both levels, i.e., five positives for the PDX-SIB method compared to three for the FDA-BAM procedure.

Ruggedness Studies

Ruggedness parameters studied were incubation times (28, 32, 46, and 50 h) and incubation temperatures (34 and 40 ± 1°C). Two positive controls (*S.* Abaetetuba and *S.* Anatum) and one negative control (*Escherichia coli*) were tested in five replicates at the 3 log CFU/mL for *Salmonella* serovars and at 7 log CFU/mL for *E. coli*. These tests were done on different days, as recommended. The results are given in Tables 4 and 5.

Lot-to-Lot Variability and Shelf-Life Stability

Lot-to-lot variability studies were done in five replicates/level of each microorganism (*S.* Abaetetuba ATCC 35640, *S.* Anatum ATCC 9270, and *E. coli* ATCC 14948) for each of the three production lots tested (Table 6). Culture dilutions of 100 µL were inoculated onto the tip of Tecra Enviroswab, which was then fully submerged into 15 mL PDX-SIB in its original sterile container and incubated at 37°C for 48 h. The results are given in Tables 6 and 7.

Discussion

PDX-SIB is an easy-to-use and interpret screening test for *Salmonella* species in environmental samples. Inclusivity and exclusivity studies revealed that PDX-SIB is comprehensive for the detection of *Salmonella* species at low levels (10–100 CFU/sample). Two strains, *S.* Cubana and *S.* Gallinarum, were originally negative in the inclusivity study. An additional isolate of *S.* Cubana was obtained from a different source, the University of Pennsylvania Salmonella Reference Center. This particular isolate was positive in fermenting the indicator compound in SIB, in contrast to the isolate obtained from the University of Minnesota's culture collection. These data suggest that the Minnesota isolate was likely defective in a metabolic pathway for fermentation of the indicator compound. With regard to specificity, high levels of some *Citrobacter* species remain a possible source of false-positive results, which, although not desired by the typical end user, tell a great deal about the overall microbial cleanliness of the areas sampled. Learning about the presence of *Citrobacter* species is also important because many occupy similar niches to *Salmonella* species and arise as contamination sources from the gastrointestinal tracts of warm-blooded animals. This information is potentially useful when monitoring food processing surfaces intended to be free of microflora after sanitation operations. These results demonstrate a cross-reaction in two of three *Citrobacter* species tested, underscoring the need to confirm all SIB-positive results by traditional biochemical or genetic methods.

PDX-SIB was found to be at least as sensitive as the reference method in all the surfaces studied. In fact, PDX-SIB was slightly more sensitive than the reference method in one of the method comparison studies: five positives for PDX-SIB, versus three for the FDA-BAM method in the stainless steel study.

Regarding the ruggedness studies, recommended parameters were studied for PDX-SIB. Results of the ruggedness studies suggested that for the most part, selected deviations from test parameters did not interfere with the true detection of microorganisms selected with the exception of *S.* Anatum, which was not detected at 40°C and 24 h incubation.

Lot-to-lot variability studies showed that there was no difference between the production lots. Shelf-life studies

Table 5. Time variability

Strain	18 h	24 h	52 h
		S. Abaetetuba	
A	+	+	+
B	+	+	+
C	+	+	+
D	+	+	+
E	+	+	+
		S. Anatum	
A	+	+	+
B	+	+	+
C	+	+	+
D	+	+	+
E	+	+	+
		E. coli	
A	–	–	–
B	–	–	–
C	–	–	–
D	–	–	–
E	–	–	–

Table 6. Lot variability

Strain	Lot No. 06007	Lot No. 06009	Lot No. 06011
S. Abaetetuba			
A	+	+	+
B	+	+	+
C	+	+	+
D	+	+	+
E	+	+	+
S. Anatum			
A	+	+	+
B	+	+	+
C	+	+	+
D	+	+	+
E	+	+	+
E. coli			
A	–	–	–
B	–	–	–
C	–	–	–
D	–	–	–
E	–	–	–

Table 7. Shelf stability

Strain	Lot No. 06007, 30 Days	Lot No. 06007, 60 Days	Lot No. 06007, 90 Days
S. Abaetetuba			
A	+	+	+
B	+	+	+
C	+	+	+
D	+	+	+
E	+	+	+
S. Anatum			
A	+	+	+
B	+	+	+
C	+	+	+
D	+	+	+
E	+	+	+
E. coli			
A	–	–	–
B	–	–	–
C	–	–	–
D	–	–	–
E	–	–	–

documented in Table 6 revealed that PDX-SIB is shelf-stable at 3 months of refrigerated storage.

In conclusion, PDX-SIB is a unique, easy-to-perform, rapid detection test for *Salmonella* species in environmental samples. The test was demonstrated to be substantially equivalent to the FDA-BAM method for the four selected surfaces and sufficiently *Salmonella*-inclusive and -exclusive, with the notable exception of some *Citrobacter* species. These studies detail the utility of PDX-SIB as a diagnostic screening test. Because PDX-SIB is a self-contained test, it minimizes cross-contamination. Furthermore, this study shows that PDX-SIB is compatible with the FDA-BAM confirmation methods, thus providing a more economical solution to *Salmonella* screening without any compromise in sensitivity. It is expected that PDX-SIB will enable more on-site *Salmonella* monitoring in environmental samples, thereby contributing to the overall goal of raising food safety standards in the processing environment.

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