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Article

MICROBIOLOGICAL METHODS

Validation of the One Broth One Plate for *Salmonella* Method for Detection of *Salmonella* Spp. in Select Food and Environmental Samples: AOAC *Performance Tested Method*^{SM} 102002

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Abstract

Background: One Broth One Plate for *Salmonella* (OBOP *Salmonella*) is a rapid and simple method for detection of *Salmonella* spp. in food and environmental samples using traditional culture methodology. The method utilizes single-step enrichment followed by plating to a selective/differential, chromogenic agar.
Objective: The purpose of the validation study was to measure the effectiveness of the OBOP *Salmonella* method in comparison to reference culture procedures.
Method: Performance of the OBOP *Salmonella* method was compared to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* Chapter 5 reference method for queso fresco, smoked salmon, cantaloupe, chocolate, black pepper, chili powder, dry pet food, and sponge samples from a stainless steel surface, or to that of the U.S. Department of Agriculture *Microbiology Laboratory Guidebook* Chapter 4.10 method for raw ground turkey, chicken carcass rinse, and pasteurized liquid egg. Inclusivity/exclusivity, robustness, and stability/lot-to-lot consistency testing was also performed.
Results: In the matrix study, there were no statistically significant differences in performance between the OBOP *Salmonella* and reference methods, as determined by probability of detection analysis ($P < 0.05$), for any of the matrixes examined. All 104 *Salmonella* spp. strains produced positive results in inclusivity testing, and all 33 non-salmonellae exclusivity strains tested negative with the OBOP *Salmonella* method.
Conclusions: Results of the validation study show that the OBOP *Salmonella* method is a reliable procedure for detection of *Salmonella* spp. in select matrixes. The method is simple to perform, requires no specialized equipment, and produces results in as little as 37 h.
Highlights: The OBOP *Salmonella* method was awarded AOAC PTM^{SM} (#102002) for detection of *Salmonella* in queso fresco, smoked salmon, cantaloupe, chocolate, black pepper, chili powder, dry pet food, sponge samples on a stainless steel

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surface, raw ground turkey, chicken carcass rinse, and pasteurized liquid egg. The method is also approved by MicroVal® for a broad range of foods under certification number 2019LR88.

Scope of Method

(a) *Analytes*.—*Salmonella enterica* and *S. bongori*.
(b) *Matrixes*.—Queso fresco, smoked salmon, cantaloupe, dry pet food, chocolate, black pepper, chili powder, raw ground turkey, chicken carcass rinse, pasteurized liquid egg, and sponge samples from stainless steel surfaces.
(c) *Summary of validated performance claims*.—No statistically significant differences in performance between the One Broth One Plate for *Salmonella* (OBOP *Salmonella*) method and the following reference methods: U.S. Food and Drug Administration *Bacteriological Analytical Manual* (FDA/BAM), Chapter 5 (1), *Salmonella*; U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (USDA/MLG), Chapter 4.10, Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges (2).

Definitions

(a) *Probability of detection (POD)*.—The proportion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. POD is concentration dependent. Several POD measures can be calculated; POD_R (reference method POD), POD_C (confirmed candidate method POD), POD_{CP} (candidate method presumptive result POD), and POD_{CC} (candidate method confirmation result POD).
(b) *Difference of probabilities of detection (dPOD)*.—Difference of probabilities of detection is the difference between any two POD values. If the confidence interval of the dPOD does not contain zero, then the difference is statistically significant at the 5% level (3, 4).

Principle

One Broth One Plate for *Salmonella* (OBOP *Salmonella*) is a rapid and simple method based on traditional culture methodology. Samples are enriched in buffered peptone water (BPW) containing a *Salmonella* selective supplement for 18 ± 2 h at 41.5 ± 1°C. After incubation, a 10 µL aliquot is streaked to Harlequin Chromogenic Agar for *Salmonella* esterase (CASE agar) and incubated for 24 ± 3 h at 37 ± 2°C. CASE agar uses a dual chromogenic system to differentiate *Salmonella* spp. from non-salmonellae. One chromogen is a target for esterase activity common to *Salmonella* spp., resulting in blue-green colonies. The second chromogen is a target for β-glucosidase activity present in some non-target organisms. Non-target organisms that are able to grow and that also possess esterase activity will form black colonies on CASE agar due to the β-glucosidase activity, masking the blue-green color formed by esterase activity. Other non-target organisms are either inhibited or form colorless colonies. Non-motile salmonellae (e.g., *Salmonella enterica* ser. Pullorum and *S. enterica* ser. Gallinarum), monophasic variants (e.g., *S. enterica* I 4,[5],12: i:-), strains with weak esterase activity (e.g., *S. enterica* ser. Dublin), lactose-positive strains (e.g., *S. enterica* subsp. *arizonae*), and serovars of *S. bongori* are all detectable on CASE agar.

Materials and Methods

Test Information

(a) *Test name*.—One Broth One Plate for *Salmonella* (OBOP *Salmonella*).
(b) *Enrichment media*.—BPW HQ (ISO), Cat. No. NCM0270.
(c) *Enrichment supplement*.—Neogen *Salmonella* Selective Supplement, Cat. No. NCM4000-10.
(d) *Selective agar*.—Harlequin® Chromogenic Agar for *Salmonella* Esterase (CASE), Cat. No. NCM1006.
(e) *Ordering information*.—In the United States: Neogen Corp., 620 Lesher Pl., Lansing, MI 48912, Tel: 800-234-5333 or 517-372-9200, Fax: 517-372-2006, Website: www.neogen.com. Outside the United States: Contact U.S. office for ordering or distributor information.

Additional Supplies and Reagents

(a) *Stomacher-type bags for sample enrichment*.—With filters, Cat. No. 6827 or equivalent.
(b) *Purified water*.
(c) *Graduated cylinder*.—250 mL, Cat. No. 9368 or equivalent.
(d) *Pipets*.—Sterile, 10 mL.
(e) *Micropipettor and tips*.—1 mL.
(f) *Petri dishes*.—Sterile.
(g) *Inoculation loops*.—10 µL, sterile.
(h) *Media and reagents as required for confirmation of presumptive Salmonella spp. colonies* (1, 2).

Apparatus

(a) *Balance*.—1 kg capacity, ± 0.1 g.
(b) *Food homogenizer*.—*Stomacher or equivalent*.
(c) *Incubators*.—Two, 41.5 ± 1°C and 37 ± 2°C.

Reference Materials

Organisms used in the validation study were obtained from the following sources: American Type Culture Collection (Manassas, VA, USA), Q Laboratories (Cincinnati, OH, USA), National Collection of Type Cultures (Salisbury, UK), U.S. Food and Drug Administration (College Park, MD, USA), and University of Pennsylvania (State College, PA, USA).

Safety Precautions

Use of this test should be restricted to individuals with appropriate laboratory training in microbiology. *Salmonella* spp. is a Biosafety Level 2 organism. Reagents are for laboratory use only. All pipetting transfers must be made using either a disposable pipet and pipetting aid or micropipettor with disposable tips. Culture media contains antimicrobial selective agents and dyes. Wear appropriate PPE and avoid contact with skin and mucous membranes. Refer to the Safety Data Sheet available from Neogen Corp. for more information. Used enrichment

cultures and agar media should be handled and disposed of as potentially infectious material. The preferred method for disposal of contaminated material is autoclaving. Items that cannot be autoclaved may be decontaminated by using a disinfectant solution, e.g., 10% household bleach, followed by rinsing with water. Consult with your facility safety director for specific instructions.

General Preparation

(a) *Use aseptic technique.*
(b) *Use filter bags for enrichment to minimize particulates.*
(c) *Do not reuse disposable supplies.*
(d) *Change pipet tips between samples.*
(e) *Avoid shaking the enrichment bag and collecting large food fragments. For fatty foods, collect the sample just below the fat layer.*
(f) *Reconstitution of Salmonella Selective Supplement.*
  (1) Add 10 mL BPW or purified water to one vial of selective supplement.
  (2) Swirl to dissolve.
(g) *Preparation of CASE agar.*
  (1) Dissolve 49.9 g CASE agar dehydrated medium in 1.0 L purified water and mix thoroughly.
  (2) Bring rapidly to a boil with frequent agitation, then temper in a water bath to 50°C.
  Note: Medium must sufficiently boil or a white precipitate will be seen in the agar. This does not affect performance and can be avoided by sufficiently boiling the medium.
  (3) Dispense into sterile Petri dishes and allow to cool.

Sample Preparation

(a) *Sample enrichment.*

(1) *25 g samples (chili powder, black pepper, queso fresco, chocolate, dry pet food, cantaloupe, smoked salmon, raw ground turkey).*—Homogenize 25 g sample in 225 mL BPW. Up to 1 h later, add 1.0 mL reconstituted *Salmonella* selective supplement. Mix thoroughly. Incubate at 41.5 ± 1°C for 18 ± 2 h.
(2) *325 g raw ground turkey samples.*—Homogenize 325 g sample in 1300 mL BPW. Add 5.8 mL reconstituted *Salmonella* selective supplement. Mix thoroughly. Incubate at 41.5 ± 1°C for 18 ± 2 h.
(3) *375 g dry pet food samples.*—Homogenize 375 g in 1875 mL BPW. Add 8.3 mL reconstituted *Salmonella* selective supplement. Mix thoroughly. Incubate at 41.5 ± 1°C for 18 ± 2 h.
(4) *100 g pasteurized liquid egg samples.*—Homogenize 100 g sample in 900 mL BPW. Add 4.0 mL reconstituted *Salmonella* selective supplement. Mix thoroughly. Incubate at 41.5 ± 1°C for 18 ± 2 h.
(5) *Chicken carcass rinse samples.*—Rinse the whole chicken carcass with 400 mL BPW following the USDA/MLG procedure (2). Remove a 30 mL aliquot of the rinse. To the 30 mL aliquot, add 30 mL BPW and 133 µL reconstituted *Salmonella* selective supplement. Mix thoroughly. Incubate at 41.5 ± 1°C for 18 ± 2 h.
(6) *Sponge samples from stainless steel surfaces.*—Add sponge sample to 100 mL BPW and add 440 µL reconstituted *Salmonella* selective supplement. Mix thoroughly. Incubate at 41.5 ± 1°C for 18 ± 2 h.

Analysis

Using a sterile inoculating loop, streak 10 µL of the enrichment culture to a CASE agar plate. A four-quadrant streak method is recommended. Incubate the plate at 37 ± 2°C for 24 ± 3 h.

Interpretation of Results

(a) Blue-green color colonies are presumptive *Salmonella* spp.
(b) Certain other *Enterobacteriaceae* will form black (e.g., *Enterobacter* spp., *Klebsiella* spp.), brown (*Proteus* spp.), or colorless (e.g., *Escherichia* spp., *Proteus* spp., *Shigella* spp.) colonies. Other organisms will be inhibited.

Confirmation

Presumptive *Salmonella* colonies may be confirmed following standard biochemical, serological, nucleic acid-based, and/or mass spectrometric procedures (1, 2).

Validation Study

The study was conducted in accordance with the current AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (3) and ISO 16140–2:2016, Microbiology of the Food Chain—Method Validation—Part 2: Protocol for the Validation of Alternative (Proprietary) Methods Against a Reference Method (5) as part of a harmonized study with MicroVal. Inclusivity/exclusivity and matrix studies were conducted to satisfy both AOAC and MicroVal requirements. In addition to inclusivity/exclusivity and matrix testing, the study also included method robustness testing and stability/lot-to-lot consistency testing. The following matrixes were tested in the method developer laboratory: chili powder, black pepper, queso fresco, chocolate, and pasteurized liquid egg. Testing of the following matrixes was conducted independently by a third-party laboratory per MicroVal requirements: smoked salmon, cantaloupe, dry pet food, raw ground turkey, chicken carcass rinse, and sponge samples from a stainless steel surface. Additional independent laboratory testing was performed by Q Laboratories and included two of the claimed matrixes (queso fresco and pasteurized liquid egg).

Method Developer Studies

Inclusivity testing

Methods.—One hundred and four *Salmonella* spp. strains were tested, representing *S. bongori,* the six recognized subspecies of *S. enterica*, and the major somatic (O) groups. Test strains were grown in BPW overnight. Two hundred and twenty-five mL BPW plus *Salmonella* selective supplement was inoculated with 10–100 CFU of the test strain and incubated at 41.5 ± 1°C for 16 h. Cultures were plated to CASE agar and incubated at 37 ± 2°C for 21 h. Presumptive *Salmonella* spp. colonies were plated to xylose lysine deoxycholate (XLD) agar and confirmed using standard methods (1, 2). The MALDI Biotyper®(Bruker, Billerica, MA, USA) was used for final confirmation (6).

Results.—Results are shown in Table 1. All 104 inclusivity strains produced blue-green colonies on CASE agar and confirmed positive as *Salmonella* spp.

Table 1. Inclusivity testing results for the OBOP Salmonella method

Species and subspecies	Serovar name	O group or antigenic formula	Source and strain No.	Origin	OBOP *Salmonella* result
S. enterica subsp. *Arizonae*		51	ATCC 13314[a]	Not available	Positive
S. enterica subsp. *Arizonae*			ATCC BAA-1577	Not available	Positive
S. enterica subsp. *Diarizonae*			ATCC BAA-639	Human feces	Positive
S. enterica subsp. *Diarizonae*		O	ATCC BAA-216	Human blood	Positive
S. enterica subsp. *Houtenae*	Halmstad	3,15; g, s, t:-	QL 024.1[b]	Clinical isolate	Positive
S. enterica subsp. *Houtenae*	Harmelen	51	ATCC 15783	Boa constrictor	Positive
S. enterica subsp. *Houtenae*	Ochsenzoll	I	ATCC 29932	Not available	Positive
S. enterica subsp. *Indica*	Ferlac	H	ATCC 43976	Not available	Positive
S. enterica subsp. *Indica*	Ferlac	H	NCTC 10458[c]	Ceylonese desiccated coconut	Positive
S. enterica subsp. *Indica*		W	ATCC BAA-1578	India	Positive
S. enterica subsp. *Salamae*	Artis	56	ATCC 700149	Not available	Positive
S. enterica subsp. *Salamae*	Basel	58	ATCC 700151	Not available	Positive
S. enterica subsp. *Enterica*	Abaetetuba	F	ATCC 35640	Creek water	Positive
S. enterica subsp. *Enterica*	Abortusequi	B	FDA 9842[d]	Not available	Positive
S. enterica subsp. *enterica*	Abortusovis	B	NCTC 10241	Not available	Positive
S. enterica subsp. *enterica*	Abony	B	NCTC 6017	Not available	Positive
S. enterica subsp. *enterica*	Adelaide	O	UPenn STS 2[e]	Not available	Positive
S. enterica subsp. *enterica*	Agona	B	ATCC 51957	Not available	Positive
S. enterica subsp. *enterica*	Agama	B	UPenn STS 3	Not available	Positive
S. enterica subsp. *enterica*	Agoueve	G	UPenn STS 5	Not available	Positive
S. enterica subsp. *enterica*	Alachua	O	UPenn STS 6	Not available	Positive
S. enterica subsp. *enterica*	Albany	C_2	UPenn STS 7	Not available	Positive
S. enterica subsp. *enterica*	Anatum	E_1	ATCC 9270	Pork liver	Positive
S. enterica subsp. *enterica*	Arkansas	E_3	UPenn STS 11	Not available	Positive
S. enterica subsp. *enterica*	Bareilly	C_1	FDA 1206H	Not available	Positive
S. enterica subsp. *enterica*	Berta	D_1	UPenn STS 13	Not available	Positive
S. enterica subsp. *enterica*	Binza	E_2	UPenn STS 14	Not available	Positive
S. enterica subsp. *enterica*	Bovismorbificans	C_2	UPenn STS 16	Not available	Positive
S. enterica subsp. *enterica*	Brandenburg	B	UPenn STS 18	Not available	Positive
S. enterica subsp. *enterica*	Bredeney	B	NCTC 5731	Not available	Positive
S. enterica subsp. *enterica*	California	B	NCTC 6018	Not available	Positive
S. enterica subsp. *enterica*	Cerro	K	UPenn STS 22	Not available	Positive
S. enterica subsp. *enterica*	Choleraesuis	C_1	ATCC 10708	Equine isolate	Positive
S. enterica subsp. *enterica*	Choleraesuis var Kunzendorf	C_1	ATCC 12011	Not available	Positive
S. enterica subsp. *enterica*	Cubana	G	UPenn STS 24	Not available	Positive
S. enterica subsp. *enterica*	Derby	B	NCTC 5721	Not available	Positive
S. enterica subsp. *enterica*	Drypool	E_1	UPenn STS 26	Not available	Positive
S. enterica subsp. *enterica*	Dublin	D_1	UPenn STS 27	Not available	Positive
S. enterica subsp. *enterica*	Eastbourne	D_1	FDA 4017H	Not available	Positive
S. enterica subsp. *enterica*	Enteritidis	D_1	ATCC 13076	Not available	Positive
S. enterica subsp. *enterica*	Galiema	C_1	QL024.2	Environmental isolate	Positive
S. enterica subsp. *enterica*	Give	E_1	UPenn STS 42	Not available	Positive
S. enterica subsp. *enterica*	Haardt	C_2	UPenn STS 44	Not available	Positive
S. enterica subsp. *enterica*	Hadar	C_2	ATCC 51956	Not available	Positive
S. enterica subsp. *enterica*	Havana	G	UPenn STS 47	Not available	Positive
S. enterica subsp. *enterica*	Heidelberg	B	ATCC 8326	Not available	Positive
S. enterica subsp. *enterica*	Illinois	E_3	ATCC 11646	Not available	Positive
S. enterica subsp. *enterica*	Indiana	B	NCTC 11304	Turkey	Positive
S. enterica subsp. *enterica*	Infantis	C_1	ATCC 51741	Pasta	Positive
S. enterica subsp. *enterica*	Javiana	D_1	ATCC 10721	Not available	Positive
S. enterica subsp. *enterica*	Jerusalem	C_1	QL024.12	Pet food	Positive
S. enterica subsp. *enterica*	Johannesburg	R	UPenn STS 56	Not available	Positive
S. enterica subsp. *enterica*	Kahla	T	ATCC 17980	Not available	Positive
S. enterica subsp. *enterica*	Kaitaan	H	QL024.7	Pet food	Positive
S. enterica subsp. *enterica*	Kentucky	C_2	ATCC 9263	Not available	Positive
S. enterica subsp. *enterica*	Krefeld	E_4	UPenn STS 58	Not available	Positive

(continued)

Table 1. (continued)

Species and subspecies	Serovar name	O group or antigenic formula	Source and strain No.	Origin	OBOP *Salmonella* result
S. enterica subsp. *enterica*	Lille	C_1	UPenn STS 59	Not available	Positive
S. enterica subsp. *enterica*	Livingstone	C_1	UPenn STS 63	Not available	Positive
S. enterica subsp. *enterica*	London	E_1	UPenn STS 64	Not available	Positive
S. enterica subsp. *enterica*	Manhattan	C_2	UPenn STS 65	Not available	Positive
S. enterica subsp. *enterica*	Mbandaka	C_1	FDA 37 N	Low moisture ingredient	Positive
S. enterica subsp. *enterica*	Menden	C_1	ATCC 15992	Feces	Positive
S. enterica subsp. *enterica*	Meleagridis	E_1	QL12074-1	Environmental isolate	Positive
S. enterica subsp. *enterica*	Minnesota	L	UPenn STS 70	Not available	Positive
S. enterica subsp. *enterica*	Montevideo	C_1	ATCC 8387	Not available	Positive
S. enterica subsp. *enterica*	Muenchen	C_2	ATCC BAA-1594	Human stool	Positive
S. enterica subsp. *enterica*	Neasden	D_1	QL024.4	Raw material	Positive
S. enterica subsp. *enterica*	Newington	E_2	QL024.8	Fish oil	Positive
S. enterica subsp. *enterica*	Newport	C_2	ATCC 6962	Food poisoning	Positive
S. enterica subsp. *enterica*	Ohio	C_1	UPenn STS 81	Not available	Positive
S. enterica subsp. *enterica*	Oranienburg	C_1	ATCC 9239	Not available	Positive
S. enterica subsp. *enterica*	Orthmarshen	C_1	QL024.13	Pet kibble	Positive
S. enterica subsp. *enterica*	Paratyphi A	A	ATCC 9150	Not available	Positive
S. enterica subsp. *enterica*	Paratyphi B	B	ATCC 10719	Not available	Positive
S. enterica subsp. *enterica*	Paratyphi C	C_1	ATCC 13428	Not available	Positive
S. enterica subsp. *enterica*	Pomona	M	ATCC 10729	Clinical isolate	Positive
S. enterica subsp. *enterica*	Poona	G	NCTC 4840	Infant enteritis	Positive
S. enterica subsp. *enterica*	Potsdam	C_1	QL15091-1A	Pet food	Positive
S. enterica subsp. *enterica*	Preston	B	QL024.16	Low moisture product	Positive
S. enterica subsp. *enterica*	Pullorum	D_1	ATCC 13036	Egg	Positive
S. enterica subsp. *enterica*	Rubislaw	F	UPenn STS 92	Not available	Positive
S. enterica subsp. *enterica*	Saintpaul	B	ATCC 9712	Cystitis	Positive
S. enterica subsp. *enterica*	Sandiego	B	UPenn STS 94	Not available	Positive
S. enterica subsp. *enterica*	Schalkwijk	H	QL024.10	Cat food	Positive
S. enterica subsp. *enterica*	Schwarzengrund	B	UPenn STS 95	Not available	Positive
S. enterica subsp. *enterica*	Senftenberg	E_4	ATCC 43845	Not available	Positive
S. enterica subsp. *enterica*	Stanley	B	ATCC 7308	Not available	Positive
S. enterica subsp. *enterica*	Sylvania	H	QL091313.4	Raw dog food	Positive
S. enterica subsp. *enterica*	Tallahassee	C_2	ATCC 12002	Not available	Positive
S. enterica subsp. *enterica*	Tennessee	C_1	QL024.6	Clinical isolate	Positive
S. enterica subsp. *enterica*	Thompson	C_1	FDA 2051H	Not available	Positive
S. enterica subsp. *enterica*	Tranoroa	55	NCTC 10252	Not available	Positive
S. enterica subsp. *enterica*	Typhi	D_1	ATCC 6539	Not available	Positive
S. enterica subsp. *enterica*	Typhimurium	B	ATCC 14028	Animal tissue	Positive
S. enterica subsp. *enterica*	Utrecht	52	NCTC 10077	Not available	Positive
S. enterica subsp. *enterica*	Urbana	N	UPenn STS 110	Not available	Positive
S. enterica subsp. *enterica*	Vellore	B	ATCC 15611	Rectal swab	Positive
S. enterica subsp. *enterica*	Virchow	C_1	ATCC 51955	Not available	Positive
S. enterica subsp. *enterica*	Volta	F	QL024.9	Raw material	Positive
S. enterica subsp. *enterica*	Westhampton	E_1	QL024.14	Dog kibble	Positive
S. enterica subsp. *enterica*	Worthington	G	UPenn STS 114	Not available	Positive
S. enterica subsp. *enterica*	Zwickau	I	NCTC 15805	Not available	Positive
S. bongori		66	ATCC 43975	Not available	Positive
S. bongori	Brookfield	66	NCTC 12419	Not available	Positive

[a] ATCC = American Type Culture Collection.
[b] QL = Q Laboratories Culture Collection.
[c] NCTC = National Collection of Type Cultures.
[d] FDA = U.S. Food and Drug Administration Culture Collection.
[e] UPenn = University of Pennsylvania Culture Collection.

Exclusivity testing

Method.—Thirty-three exclusivity strains were tested, comprised primarily of non-*Salmonella* members of the *Enterobacteriaceae*. Test strains were grown in brain heart infusion broth at 37 ± 1°C for 16 h. Dilutions were made and BPW was inoculated at a level of approximately 1×10^5 CFU/mL, followed by incubation at 41.5 ± 1°C for 21 h. Cultures were plated to CASE agar and incubated at 37 ± 2°C for 21 h.

Results.—Results are presented in Table 2. None of the test strains produced presumptive (blue-green) colonies on CASE agar, therefore results were negative for all 33 exclusivity strains.

Table 2. Exclusivity testing results for the OBOP *Salmonella* method

Organism	Source and strain No.	Origin	OBOP *Salmonella* result
Aeromonas hydrophila	ATCC 49140[a]	Clinical Isolate	Negative—No growth
Alcaligenes faecalis	ATCC 8750	Not available	Negative—No growth
Bacillus subtilis	ATCC 6051	Not available	Negative—No growth
Campylobacter jejuni	ATCC 33560	Feces, bovine	Negative—No growth
Candida albicans	ATCC 24433	Nail infection	Negative—Colorless colonies
Carnobacterium maltaromaticum	ATCC 43224	Vacuum-packed beef	Negative—No growth
Citrobacter youngae	ATCC 11102	Not available	Negative—Black colonies
Citrobacter farmer	ATCC 51112	Feces, human	Negative—Black colonies
Citrobacter freundii	ATCC 8090	Not available	Negative—Black colonies
Edwardsiella tarda	ATCC 15947	Feces, human	Negative—No growth
Enterobacter aerogenes	ATCC 13048	Sputum	Negative—Black colonies
Enterobacter cloacae	ATCC 23355	Not available	Negative—Black colonies
Enterococcus faecalis	ATCC 29212	Urine	Negative—No growth
Escherichia coli	ATCC 25922	Clinical isolate	Negative—Colorless colonies
Escherichia hermannii	ATCC 33651	Arm wound	Negative—Colorless colonies
Hafnia alvei	ATCC 51813	Milk	Negative—Colorless colonies
Lactobacillus kefiri	ATCC 35411	Kefir	Negative—No growth
Lactobacillus lactis	ATCC 4794	Not available	Negative—No growth
Listeria monocytogenes	ATCC 7644	Human isolate	Negative—No growth
Kluyvera intermedia	ATCC 33110	Surface water	Negative—Black colonies
Klebsiella pneumonia	ATCC 13883	Not available	Negative—Black colonies
Kocuria rhizophila	ATCC 9341	Soil	Negative—No growth
Morganella morganii	ATCC 25829	Human	Negative—Colorless colonies
Proteus mirabilis	ATCC 7002	Urine	Negative—No growth
Proteus vulgaris	ATCC 6380	Clinical isolate	Negative—No growth
Pseudomonas aeruginosa	ATCC 27853	Clinical isolate	Negative—No growth
Serratia marcescens	ATCC 14756	Fort Detrick, MD	Negative—Black colonies
Serratia marcescens	ATCC 13880	Human	Negative—Black colonies
Shigella sonnei	ATCC 29930	Not available	Negative—No growth
Shigella boydii	ATCC 9207	Pork liver	Negative—No growth
Staphylococcus aureus	ATCC 10832	Not available	Negative—No growth
Staphylococcus epidermidis	ATCC 12228	Not available	Negative—No growth
Streptococcus pneumoniae	ATCC 6302	Not available	Negative—No growth

[a] ATCC = American Type Culture Collection.

Matrix testing

Methods.—For chili powder, black pepper, queso fresco, smoked salmon, cantaloupe, chocolate, dry pet food, and sponge samples from a stainless steel surface, performance of the OBOP *Salmonella* method was compared to that of the FDA/BAM reference culture procedure (1). For raw ground turkey, chicken carcass rinse, and liquid pasteurized egg, performance of the OBOP *Salmonella* method was compared to that of the USDA/MLG reference culture procedure (2). Food items and chicken carcasses used for rinse testing were obtained from local suppliers.

Dilutions of overnight cultures were used as inocula. Test strains (Table 3) were taken from frozen stocks and grown on tryptic soy agar (TSA) with 5% sheep blood at 35 ± 1°C for 24 ± 2 h. A single colony was then transferred to BPW and incubated for 35 ± 1°C for 24 ± 2 h, after which appropriate dilutions were made in BPW.

For heat-processed foods (smoked salmon, queso fresco, liquid pasteurized egg), the *Salmonella* spp. inoculum was heated at 50–55°C for 5–10 min and the degree of injury was assessed by plating to non-selective (TSA) and selective (XLD) media. For all food matrixes other than dry pet food, black pepper, chili powder, and chicken carcass rinse, the liquid inoculum was applied to the matrix in bulk, followed by extensive manual mixing and division into individual test portions. Chocolate was melted, the inoculum mixed in, and then allowed to re-solidify. Chicken carcasses were inoculated by applying 1 mL of liquid inoculum to the carcass cavity. For low-moisture food matrixes (dry pet food, black pepper, chili powder), bulk product was inoculated with a lyophilized cell pellet of the test strain. After equilibration, the seeded material was mixed with additional, uninoculated material to achieve the desired contamination level. Low moisture matrixes (and controls) were allowed to equilibrate for 14 days at 20–25°C before analysis. All other matrixes were allowed to equilibrate for 48–72 h at 2–8°C.

For all food matrixes except chicken carcasses, a most probable number (MPN) analysis was conducted on the inoculated and control matrixes on the day of analysis to determine the level of surviving salmonellae. The MPN analysis was performed in accordance with the AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, Annex A (3), and included use of an MPN calculator to determine MPN values and confidence intervals (7). For chicken carcasses, the inoculation level was determined by performing a standard plate count on the inoculum culture.

For stainless steel, 10 × 10 cm surface areas were inoculated with a culture dilution. In addition to the *Salmonella* spp. target strain, the inoculum included the competitor organism *Citrobacter freundii* at a level approximately 10-fold higher than that of the *Salmonella* inoculum. Standard plate counts were performed on the inoculum cultures to determine the actual levels. The inoculum was allowed to dry for 16–24 h at 20–25°C. Surface

Table 3. Method comparison results: OBOP *Salmonella* presumptive versus confirmed

Matrix	Strain	Level (CFU/portion)[a]	N[b]	OBOP *Salmonella* presumptive			OBOP *Salmonella* confirmed				
				x[c]	POD_{CP}[d]	95% CI	x	POD_{CC}[e]	95% CI	$dPOD_{CP}$[f]	95% CI[g]
Chili powder	*Salmonella* Oranienberg	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 9239[h]	0.47 (0.25, 0.78)	30	6	0.20	0.10, 0.37	6	0.20	0.10, 0.37	0	−0.09, 0.09
		54 (18, 156)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Black pepper	*Salmonella* Weltevreden	–	5	0	0	0, 0.43	0	0	0. 0.43	0	−0.47, 0.47
	CDC 147[i]	0.43 (0.21, 0.71)	30	10	0.33	0.19, 0.51	10	0.33	0.19, 0.51	0	−0.09, 0.09
		116 (25, 537)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Queso fresco	*Salmonella*	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.47, 0.47
	Agona	1.1 (0.71, 1.8)	30	14	0.47	0.30, 0.64	14	0.47	0.30, 0.64	0	−0.09, 0.09
	CDC 1201–82	6.0 (1.7, 22)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Queso fresco[j]	*Salmonella*	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	Agona	0.45 (0.22, 0.78)	20	9	0.45	0.26, 0.66	9	0.45	0.26, 0.66	0	−0.13, 0.13
	ATCC 51957	2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Chocolate	*Salmonella* Senftenberg	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 8400	1.3 (0.85, 2.0)	30	17	0.57	0.39, 0.73	17	0.57	0.39, 0.73	0	−0.09, 0.09
		6.0 (1.7, 22)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Dry pet food, 25 g[k]	*Salmonella* Othmarschen	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.47, 0.47
	QL 024.16[l]	0.70 (0.42, 1.1)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	−0.13, 0.13
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Dry pet food, 375 g[k]	*Salmonella* Othmarschen	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.47, 0.47
	QL 024.16	0.70 (0.42, 1.1)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	−0.13, 0.13
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Cantaloupe[k]	*Salmonella*	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	Newport	0.55 (0.29, 0.93)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	−0.13, 0.13
	ATCC 6962	3.7 (1.0, 9.0)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Smoked salmon[k]	*Salmonella* Montevideo	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 8387	0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	−0.13, 0.13
		2.0 (0.91, 4.3)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Raw ground turkey, 25 g[k]	*Salmonella* Typhimurium	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 14028	1.0 (0.61, 1.7)	20	14	0.70	0.48, 0.85	14	0.70	0.48, 0.85	0	−0.13, 0.13
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Raw ground turkey, 325 g[k]	*Salmonella* Typhimurium	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 14028	1.0 (0.61, 1.7)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	−0.13, 0.13
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Pasteurized liquid egg	*Salmonella* Enteritidis	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 13076	0.35 (0.14, 0.62)	30	9	0.30	0.17, 0.48	9	0.30	0.17, 0.48	0	−0.09, 0.09
		43 (10, 186)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Pasteurized liquid egg[j]	*Salmonella* Enteritidis	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 13076	0.75 (0.44, 1.2)	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0	−0.13, 0.13
		3.7 (1.0, 9.0)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Chicken carcass rinse[k]	*Salmonella* Kentucky	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 9263	26[m]	20	10	0.50	0.30, 0.70	10	0.50	0.30, 0.70	0	−0.13, 0.13
		54[m]	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Stainless steel[k]	*Salmonella* Derby	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	NCTC5721	59/720[o]	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	−0.13, 0.13
	+ *Citrobacter freundii*[n] ATCC 8090	240/3100[o]	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47

[a] From MPN analysis.
[b] N = Number of test portions.
[c] x = Number of positive test portions.
[d] POD_{CP} = Candidate method presumptive positive outcomes divided by the total number of trials.
[e] POD_{CC} = Candidate method confirmed positive outcomes divided by the total number of trials.
[f] $dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values.
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] ATCC =American Type Culture Collection, Manassas, VA.
[i] CDC = Centers for Disease Control and Prevention, Atlanta, GA.
[j] Trial performed by the independent laboratory.
[k] Trial performed by an independent third-party laboratory as part of the MicroVal study.
[l] QL = Q Laboratories, Cincinnati, OH.
[m] CFU per carcass, determined by plate count of the inoculum culture.
[n] NCTC = National Collection of Type Cultures, Salisbury, UK.
[o] CFU per test area, determined by plate count of the inoculum culture.
'–' means samples were not spiked with Salmonella.

areas were sampled with sponges following standard procedures.

For each matrix, 40 (or in some cases 60) test portions were prepared at an inoculation level (0.2–2 CFU/test portion) intended to produce a fractional positive data set (25–75% of test portions positive). Ten test portions were prepared at a level of approximately 2–10 CFU/test portion; this level is expected to produce 100% positive results. Ten uninoculated test portions were prepared as controls. For this unpaired study, from each group, half of the test portions were tested by the OBOP *Salmonella* method and half by the appropriate reference method. In the case of 325 g (raw ground turkey) and 375 g (dry pet food) test portions, test portions were prepared by mixing 25 g of inoculated product with an additional 300 or 350 g of product.

The OBOP *Salmonella* method was performed as described in *Materials and Methods*. All OBOP *Salmonella* method enrichment cultures were subject to confirmation following the relevant reference method procedures (including secondary enrichment, if applicable), regardless of whether they yielded presumptive positive colonies on CASE agar. Reference methods were performed as described in the current versions of the published procedures (1, 2).

POD analysis at $P < 0.05$ (3, 4) was used to determine if differences in the number of positive results between methods was significant. OBOP *Salmonella* presumptive and confirmed results were compared using a paired POD model and OBOP *Salmonella* final and reference method results were compared using an unpaired POD model.

Results.—Results are shown in Tables 3 and 4. The desired fractional data sets for the low-level inoculated test portions were obtained for all matrixes. All presumptive positive OBOP *Salmonella* results were confirmed; there were no false-positive results in the study (Table 3). In the 13 trials encompassing 10 foods and 1 environmental surface, there were no significant differences in the number of positive results obtained with the OBOP *Salmonella* and reference culture methods (Table 4). For all matrixes, all high-level inoculated test portions produced confirmed positive results by both methods, and all uninoculated control test portions produced negative results by both methods.

Robustness testing

Methods.—Robustness of the OBOP *Salmonella* method was assessed in a nine-condition matrix experiment with variations introduced to three method parameters (Table 5). Method variables examined included: (1) enrichment culture incubation temperature, (2) CASE agar incubation time, and (3) CASE agar incubation temperature. Queso fresco inoculated with *S. enterica* ser. Agona was used as the test matrix. Results from the nine conditions were compared by POD analysis at $P < 0.05$ to determine if any conditions with variables produce results significantly different from those of the standard condition.

Results.—Results are shown in Table 5. For the positive samples, there were no significant differences in the number of positive results obtained with any of the eight conditions with variations to the normal method operating parameters compared with the nominal condition, as determined by unpaired POD analysis at $P < 0.05$. The largest difference was with condition five, where one positive result was obtained in comparison to five positive results for the nominal condition [(dPOD = −0.40 (−0.68, 0.00)]. For the negative samples, all conditions produced 100% negative results except for condition two (three positive results) and condition four (one positive result). The differences between these results and those of the nominal condition are not significant by POD analysis; for condition two versus the nominal condition, dPOD = 0.30 (−0.04, 0.60). The cause of the unexpected positive results on negative samples is not known, but they could have resulted from accidental contamination in the laboratory due to the large number of samples being processed.

Stability and lot-to-lot consistency testing

Methods.—A study was conducted to establish the stability of CASE agar as the dehydrated medium of 2 years when stored at 2–8°C. The agar was tested at time 0, stored at 30°C for 1, 2, 3, 4, and 5 days (to simulate shipping conditions), stored for 2 years at 2–8°C, then re-tested. All testing points included three *Salmonella* strains and five exclusive strains.

An accelerated stability study of the *Salmonella* selective supplement in vials was conducted to establish stability of 1 year when stored at 2–8°C. Vials were held at 37°C for periods of time to simulate storage at 2–8°C for 3, 6, 9, and 12 months. At all time points, the supplement was tested for successful recovery of three *Salmonella* serovars on CASE agar, and additionally for recovery of a single *Salmonella* serovar in the presence of a large excess of competing bacteria.

A lot-to-lot consistency study with OBOP *Salmonella* was performed on three manufactured lots, including three lots of BPW, three lots of selective supplement, and three lots of CASE agar. Queso fresco served as the test matrix. Product was inoculated in bulk with *S. enterica* ser. Agona at a level expected to produce a fractional positive data set. A second bulk portion of product was inoculated at a level 5 to 10-fold higher, a level expected to produce 100% positive results. Additional product served as uninoculated control material. At each level (fractional, high, and control), 10 25 g test portions were prepared and enriched in accordance with the test instructions. Each of the 30 test portions were tested with the OBOP *Salmonella* method. For each method component and at each level, paired POD analyses at $P < 0.05$ were performed to determine if there were significant differences in the number of positive results obtained between the three lots.

Results.—For the CASE agar stability study, no obvious differences in performance were noted comparing the initial and final time points for either the control sample or for any of the media samples held at elevated temperature for 1–5 days (data not shown). Stability of 2 years has been established for CASE agar dehydrated medium when stored at 2–8°C.

For the *Salmonella* selective supplement stability study, recovery of the three *Salmonella* serovars was consistent in all test samples throughout the time course of the study, as was recovery of *S. enterica* ser. Virchow in the presence of high levels of competing bacteria (data not shown). Stability of 1 year has been established for the *Salmonella* selective supplement when stored in vials at 2–8°C.

BPW HQ (ISO) stability is based on the shelf life of the standard, 4 years from manufacture.

In the lot-to-lot consistency trial, for the low-level samples, lots 1, 2, and 3 produced 70, 70, and 80% positive results, respectively. The difference between results for lots 1 and 2 versus lot 3 is not significant by POD analysis [dPOD = −0.10 (−0.44, 0.26)]. All high-level samples produced positive results and all negative control samples produced negative results for all lots.

Table 4. Method comparison results: OBOP Salmonella confirmed vs. reference method

Matrix	Strain	Level (CFU/portion)[a]	N[b]	OBOP *Salmonella* Confirmed			Reference Method			dPOD$_C^f$	95% CI[g]
				x[c]	POD$_C^d$	95% CI	x	POD$_R^e$	95% CI		
Chili powder	*Salmonella* Oranienberg	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.43, 0.43
	ATCC 9239[h]	0.47 (0.25, 0.78)	30	6	0.20	0.10, 0.37	10	0.33	0.19, 0.51	−0.13	−0.34, 0.09
		54 (18, 156)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Black pepper	*Salmonella* Weltevreden	–	5	0	0	0, 0.43	0	0	0. 0.43	0	−0.43, 0.43
	CDC 147[i]	0.43 (0.21, 0.71)	30	10	0.33	0.19, 0.51	10	0.33	0.19, 0.51	0	−0.23, 0.23
		116 (25, 537)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Queso fresco	*Salmonella*	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	Agona	1.1 (0.71, 1.8)	30	14	0.47	0.30, 0.64	21	0.70	0.52, 0.83	−0.23	−0.44, 0.01
	CDC 1201-82	6.0 (1.7, 22)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Queso fresco[j]	*Salmonella*	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.43, 0.43
	Agona	0.45 (0.22, 0.78)	20	9	0.45	0.26, 0.66	7	0.35	0.18, 0.57	0.10	−0.19, 0.37
	ATCC 51957	2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Chocolate	*Salmonella* Senftenberg	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 8400	1.3 (0.85, 2.0)	30	17	0.57	0.39, 0.73	22	0.73	0.56, 0.86	−0.17	−0.38, 0.07
		6.0 (1.7, 22)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Dry pet food, 25 g[k]	*Salmonella* Othmarschen	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	QL 024.16[l]	0.70 (0.42, 1.1)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	−0.23, 0.32
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Dry pet food, 375 g[k]	*Salmonella* Othmarschen	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	QL 024.16	0.70 (0.42, 1.1)	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	−0.23, 0.32
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Cantaloupe[k]	*Salmonella*	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	Newport	0.55 (0.29, 0.93)	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	−0.15, 0.41
	ATCC 6962	3.7 (1.0, 9.0)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Smoked salmon[k]	*Salmonella* Montevideo	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	ATCC 8387	0.50 (0.25, 0.86)	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0	−0.28, 0.28
		2.0 (0.91, 4.3)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Raw ground turkey, 25 g[k]	*Salmonella* Typhimurium	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	ATCC 14028	1.0 (0.61, 1.7)	20	14	0.70	0.48, 0.85	11	0.55	0.34, 0.74	0.15	−0.14, 0.41
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Raw ground turkey, 325 g[k]	*Salmonella* Typhimurium	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	ATCC 14028	1.0 (0.61, 1.7)	20	11	0.55	0.34. 0.74	11	0.55	0.34, 0.74	0	−0.28, 0.28
		2.6 (1.1, 5.8)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Pasteurized liquid egg	*Salmonella* Enteritidis	–	5	0	0	0, 0.43	0	0	0, 0.43	0	−0.47, 0.47
	ATCC 13076	0.35 (0.14, 0.62)	30	9	0.30	0.17, 0.48	13	0.43	0.27, 0.61	−0.13	−0.35, 0.11
		43 (10, 186)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.47, 0.47
Pasteurized liquid egg[j]	*Salmonella* Enteritidis	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	ATCC 13076	0.75 (0.44, 1.2)	20	11	0.55	0.34, 0.74	8	0.40	0.22, 0.61	0.15	−0.15, 0.41
		3.7 (1.0, 9.0)	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Chicken carcass rinse[k]	*Salmonella* Kentucky	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	ATCC 9263	26[m]	20	10	0.50	0.30, 0.70	6	0.30	0.15, 0.52	0.20	−0.10, 0.45
		54[m]	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43
Stainless steel[k]	*Salmonella* Derby	–	5	0	0	0, 0.43	5	0	0, 0.43	0	−0.43, 0.43
	NCTC5721 + *Citrobacter freundii*[n] ATCC 8090	59/720[o]	20	8	0.40	0.22, 0.61	7	0.35	0.18, 0.57	0.05	−0.23, 0.32
		240/3100[o]	5	5	1	0.57, 1	5	1	0.57, 1	0	−0.43, 0.43

[a] From MPN analysis.
[b] N = Number of test potions.
[c] x = Number of positive test portions.
[d] POD$_C$ = Candidate method confirmed positive outcomes divided by the total number of trials.
[e] POD$_R$ = Reference method confirmed positive outcomes divided by the total number of trials.
[f] dPOD$_C$ = Difference between the candidate method and reference method POD values.
[g] 95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
[h] ATCC =American Type Culture Collection, Manassas, VA.
[i] CDC = Centers for Disease Control and Prevention, Atlanta, GA.
[j] Trial performed by the independent laboratory.
[k] Trial performed by an independent third-party laboratory as part of the MicroVal study.
[l] QL = Q Laboratories, Cincinnati, OH.
[m] CFU per carcass, determined by plate count of the inoculum culture.
[n] NCTC = National Collection of Type Cultures, Salisbury, UK.
[o] CFU per test area, determined by plate count of the inoculum culture.
'–' means samples were not spiked with Salmonella.

Table 5. Results of robustness testing for the OBOP *Salmonella* method

Condition	Enrichment incubation temperature, °C	CASE agar i ncubation time, h	CASE agar incubation temperature, °C	Positive results, %[a]	
				Negative sample[b]	Positive sample[c]
1	40.5	21	35	0	50
2	40.5	21	39	30	20
3	42.5	21	35	0	20
4	42.5	21	39	10	70
5	40.5	27	35	0	10
6	40.5	27	39	0	60
7	42.5	27	35	0	50
8	42.5	27	39	0	20
9[d]	41.5	24	37	0	50

[a] 10 replicates tested.
[b] Enriched uninoculated queso fresco.
[c] Queso fresco inoculated with *S. enterica* ser. Agona at ~0.5–1.0 CFU/test portion.
[d] Standard conditions for the OBOP *Salmonella* method.

Independent Laboratory Testing

Method. Independent laboratory testing was performed with two matrixes (queso fresco and pasteurized liquid egg) to verify results obtained in the method developer laboratory. Procedures for analysis were consistent with those of the method developer testing.

Results.—Results are shown in Tables 3 and 4. For queso fresco, there were nine presumptive positive results by the OBOP *Salmonella* method, and all were confirmed. There were seven positive results by the FDA/BAM reference method. This difference was not significant by POD analysis at $P < 0.05$. For pasteurized liquid egg, there were 11 presumptive positive results by the OBOP *Salmonella* method, and all were confirmed. There were eight positive results by the USDA/MLG reference method. Again, this difference was not significant by POD analysis at $P < 0.05$. Results of independent laboratory testing verify those of the method developer laboratory for these two matrixes.

Discussion

Results of the validation study demonstrate that the OBOP *Salmonella* method is an effective procedure for detection of *Salmonella* spp. in select food and environmental samples. Sensitivity is equivalent to that of the FDA/BAM and USDA/MLG reference culture methods as determined by probability of detection analysis. Overall, in 15 trials with 10 foods and one environmental surface, there were 229 positive results (all confirmed) by the OBOP *Salmonella* method versus 231 positive results by the reference culture procedures. Specificity of the OBOP *Salmonella* method was 100%; there were no false-positive results. Inclusivity and exclusivity were both 100%. Results of robustness testing showed that the method can withstand modest perturbations to multiple operating parameters simultaneously and still produce accurate results.

The OBOP *Salmonella* method requires only traditional culture media. No diagnostic test kits or specialized equipment are needed. The method is simple to perform and provides results in as little as 37 h.

Conclusions

Based on results of the validation study reported herein, the OBOP *Salmonella* method was granted *Performance Tested Method* status by AOAC INTERNATIONAL (PTM # 102002).

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References

1. U.S. Food and Drug Administration (2019) *Bacteriological Analytical Manual*, Chapter 5, *Salmonella*, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella (accessed December 2019)
2. U.S. Department of Agriculture, Food Safety Inspection Service (2019) *Microbiology Laboratory Guidebook*, Chapter 4.10, Isolation and identification of *Salmonella* from meat, poultry, pasteurized egg, and siluriformes (fish) products and carcass and environmental sponges, https://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/mlg-4.pdf? MOD=AJPERES (accessed January 2020)
3. Official Methods of Analysis (2016) 20th Ed., Appendix J: AOAC INTERNATIONAL *Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces*,

AOAC INTERNATIONAL, Rockville, MD. http://www.eoma.aoac.org/app_j.pdf
4. Wehling, P., LaBudde, R.A., Brunelle, S.L., & Nelson, M.T. (2011) *J. AOAC Int.* **94**, 335–347
5. ISO 16140-2:2016: Microbiology of the Food Chain—Method Validation—Part 2: Protocol for the Validation of Alternative (Proprietary) Methods Against a Reference Method, Brussels, Belgium
6. Bastin, B., Bird, P., Benzinger, M.J., Crowley, E., Agin, J., Goins, D., Sohier, D., Timke, M., Shi, G., & Kostrzewa, M. (2018) *J. AOAC Int.* **101**, 1593–1609
7. Least Cost Formulations, Ltd., MPN Calculator, Version 1.6