Validation of the One Broth One Plate for *Salmonella* Method for Detection of *Salmonella* spp. in Select Food and Environmental Samples: AOAC Performance

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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.

Methods: 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.

Principle of the Method

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 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 Salmonella enterica ser. Gallinarum), monophasic variants (e.g., S. enterica I 4,[5],12:i-), strains with weak esterase activity (e.g., Salmonella enterica ser. Dublin), lactose-positive strains (e.g.,
Salmonella enterica subsp. arizonae), and serovars of Salmonella bongori are all detectable on CASE agar.

General Information

Methods for detection of Salmonella spp. in food and environmental samples are based on traditional microbiological culture techniques or more recent technologies such as immunoassay, nucleic acid hybridization, PCR, and several others. Many of these methods produce results in 24 h or less, but require complex, expensive instrumentation and highly skilled operators. There is a need for methods that can provide rapid results yet be easily performed in laboratories without the need for specialized equipment.

Scope of method

(a) Analytes.—Salmonella enterica and Salmonella 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 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; $\text{POD}_R$ (reference method POD), $\text{POD}_C$ (confirmed candidate method POD), $\text{POD}_{\text{CP}}$ (candidate method presumptive result POD) and $\text{POD}_{\text{CC}}$ (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).

**Materials and Methods**

**Test information**

(a) **Test name.**—One Broth One Plate for *Salmonella* (OBOP Salmonella).

(b) **Enrichment Media.**—Buffered Peptone Water 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](http://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 Buffered Peptone Water 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 buffered peptone water. 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 1,300 mL buffered peptone water. 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 1,875 mL buffered peptone water. 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 buffered peptone water. 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 buffered peptone water following the USDA/MLG procedure (2). Remove a 30 mL aliquot of the rinse. To the 30 mL aliquot, add 30 mL buffered peptone water 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 buffered peptone water and add 440 µL reconstituted Salmonella selective supplement. Mix thoroughly. Incubate at 41.5 ± 1°C for 18 ± 2 h.

Analysis
(a) 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

(a) 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 International 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 (Cincinnati, OH, USA) and included two of the claimed matrixes (queso fresco and pasteurized liquid egg).

Method Developer Studies

Inclusivity Testing

(a) Methods.—One hundred 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 buffered peptone water overnight. Two hundred twenty-five mL buffered peptone water 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).

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

Exclusivity Testing

(a) Methods.—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 buffered peptone water was inoculated at a level of approximately 1 x 10⁵ 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.

(b) 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.
Matrix Testing

(a) 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 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 cm x 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 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 g 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).
Probability of detection (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.

(b) *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**

(a) *Methods.*—Robustness of the OBOP *Salmonella* method was assessed in a 9-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 *Salmonella enterica* ser. Agona was used as the test matrix. Results from the 9 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.

(b) *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 8 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 5, where one positive result was
obtained in comparison to 5 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 2 (3 positive results) and condition 4 (1 positive result). The differences between these results and those of the nominal condition are not significant by POD analysis; for condition 2 vs. 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

(a) 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 3 Salmonella strains and 5 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, 3 lots of selective supplement, and 3 lots of CASE agar. Queso fresco served as the test matrix. Product was inoculated in bulk with Salmonella 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 3 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 *Salmonella 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.

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 vs. lot 3 is not significant by POD analysis (\( d\text{POD} = -0.10 \ (-0.44, 0.26) \)). All high-level samples produced positive results and all negative control samples produced negative results for all lots.

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

(b) **Results.**—Results are shown in Tables 3 and 4. For queso fresco, there were 9 presumptive positive results by the OBOP *Salmonella* method, and all were confirmed. There were 7 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 8 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 One Broth One Plate for *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 vs. 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, it is recommended that the One Broth One Plate for *Salmonella* method by granted *Performance Tested Method* status by AOAC International.

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| Species and subspecies                  | Serovar Name | O group or Antigenic Formula | Source and Strain No. | Origin          | OBOP Salmonella Result |
|----------------------------------------|--------------|------------------------------|-----------------------|-----------------|------------------------|
| S. enterica subsp. arizonae            |              |                              |                       |                 |                        |
|                                        |              |                              | ATCC® 13314           | 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:-                 | QLb 024.1             | 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            | Ceylonese desiccated coconut | Positive |
| S. enterica subsp. indica              |              | W                            | ATCC BAA-1578         | India           | Positive               |
| S. enterica subsp. salamae             | Artis        |                              | 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             | 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          | 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<sub>2</sub>                | UPenn STS 7           | Not Available   | Positive               |
| S. enterica subsp. enterica            | Anatum       | E<sub>1</sub>                | ATCC 9270             | Pork liver      | Positive               |
| S. enterica subsp. enterica            | Arkansas     | E<sub>3</sub>                | UPenn STS 11          | Not Available   | Positive               |
| S. enterica subsp. enterica            | Bareilly     | C<sub>1</sub>                | FDA 1206H             | Not Available   | Positive               |
| Strain Name                  | Subsp. subsp. enterica | Reference Code | Availability | Result   |
|-----------------------------|------------------------|----------------|--------------|----------|
| S. enterica                 |                        |                |              |          |
| Berta                       |                        | UPenn STS 13   | Not Available| Positive |
| Binza                       |                        | UPenn STS 14   | Not Available| Positive |
| Bovismorbificans            |                        | UPenn STS 16   | Not Available| Positive |
| Brandenburg                 |                        | UPenn STS 18   | Not Available| Positive |
| Bredeney                    |                        | NCTC 5731      | Not Available| Positive |
| California                  |                        | NCTC 6018      | Not Available| Positive |
| Cerro                       |                        | UPenn STS 22   | Not Available| Positive |
| Choleraesuis                |                        | ATCC 10708     | Equine isolate| Positive |
| Choleraesuis var Kunzendorf |                        | ATCC 12011     | Not Available| Positive |
| Cubana                      |                        | UPenn STS 24   | Not Available| Positive |
| Derby                       |                        | NCTC 5721      | Not Available| Positive |
| Drypool                     |                        | UPenn STS 26   | Not Available| Positive |
| Dublin                      |                        | UPenn STS 27   | Not Available| Positive |
| Eastbourne                  |                        | FDA 4017H      | Not Available| Positive |
| Enteritidis                 |                        | ATCC 13076     | Not Available| Positive |
| Galiema                     |                        | QL024.2        | Environmental isolate | Positive |
| Give                        |                        | UPenn STS 42   | Not Available| Positive |
| Haardt                      |                        | UPenn STS 44   | Not Available| Positive |
| Hadar                       |                        | ATCC 51956     | Not Available| Positive |
| Havana                      |                        | UPenn STS 47   | Not Available| Positive |
| Heidelberg                  |                        | ATCC 8326      | Not Available| Positive |
| Illinois                    |                        | ATCC 11646     | Not Available| Positive |
| Indiana                     |                        | ATCC 11304     | Turkey       | Positive |
| Infantis                    |                        | ATCC 51741     | Pasta        | Positive |
| Javiana                     |                        | ATCC 10721     | Not Available| Positive |
| Jerusalem                   |                        | QL024.12       | Pet food     | Positive |
| Johannesburg                |                        | UPenn STS 56   | Not Available| Positive |
| Kahla                       |                        | ATCC 17980     | Not Available| Positive |
| Species          | Strain Name  | Characteristic          | Source | Result |
|------------------|--------------|-------------------------|--------|--------|
| S. enterica subsp. enterica | Kaitaan     | H                      | QL024.7 | Pet food | Positive |
| S. enterica subsp. enterica | Kentucky    | C₂                    | ATCC 9263 | Not Available | Positive |
| S. enterica subsp. enterica | Krefeld     | E₄                    | UPenn STS 58 | Not Available | Positive |
| S. enterica subsp. enterica | Lille       | C₁                    | UPenn STS 59 | Not Available | Positive |
| S. enterica subsp. enterica | Livingstone | C₁                    | UPenn STS 63 | Not Available | Positive |
| S. enterica subsp. enterica | London      | E₁                    | UPenn STS 64 | Not Available | Positive |
| S. enterica subsp. enterica | Manhattan   | C₂                    | UPenn STS 65 | Not Available | Positive |
| S. enterica subsp. enterica | Mbandaka    | C₁                    | FDA 37N | Low moisture ingredient | Positive |
| S. enterica subsp. enterica | Menden      | C₁                    | ATCC 15992 | Environmental isolate | Positive |
| S. enterica subsp. enterica | Meleagrisid | E₁                    | QL12074-1 | Feces | Positive |
| S. enterica subsp. enterica | Minnesota   | L                      | UPenn STS 70 | Not Available | Positive |
| S. enterica subsp. enterica | Montevideo  | C₁                    | ATCC 8387 | Not Available | Positive |
| S. enterica subsp. enterica | Muenchen    | C₂                    | ATCC BAA-1594 | Human stool | Positive |
| S. enterica subsp. enterica | Neasden     | D₁                    | QL024.4 | Raw material | Positive |
| S. enterica subsp. enterica | Newington   | E₂                    | QL024.8 | Fish oil | Positive |
| S. enterica subsp. enterica | Newport     | C₂                    | ATCC 6962 | Food poisoning | Positive |
| S. enterica subsp. enterica | Ohio        | C₁                    | UPenn STS 81 | Not Available | Positive |
| S. enterica subsp. enterica | Oranienburg | C₁                    | ATCC 9239 | Not Available | Positive |
| S. enterica subsp. enterica | Orthmarshen | C₁                    | 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 | C₁                    | 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     | C₁                    | QL15091-1A | Pet food | Positive |
| S. enterica subsp. enterica | Preston     | B                     | QL024.16 | Low moisture product | Positive |
| S. enterica subsp. enterica | Pullorum    | D₁                    | ATCC 13036 | Egg | Positive |
| S. enterica subsp. enterica | Rubislaw    | F                     | UPenn STS 92 | Not Available | Positive |
| **S. enterica subsp. enterica** | **Location**     | **Code** | **Strain Type** | **Source**  | **Sample** | **Availability** | **Result** |
|--------------------------------|-----------------|----------|----------------|-------------|------------|-----------------|-----------|
| Saint Paul                    |                 | B        | ATCC 9712      | Cystitis    |            | Positive        |
| Sandiego                      |                 | B        | UPenn STS 94   | Not Available |           | Positive        |
| Schalkwijk                    |                 | H        | QL024.10       | Cat food    |            | Positive        |
| Schwarzengründ                |                 | B        | UPenn STS 95   | Not Available |           | Positive        |
| Senftenberg                   |                 | E4       | ATCC 43845     | Not Available |           | Positive        |
| Stanley                       |                 | B        | ATCC 7308      | Not Available |           | Positive        |
| Sylvania                      |                 | H        | QL091313.4     | Raw dog food |            | Positive        |
| Tallahassee                   |                 | C2       | ATCC 12002     | Not Available |           | Positive        |
| Tennessee                     |                 | C1       | QL024.6        | Clinical isolate |     | Positive        |
| Thompson                      |                 | C1       | FDA 2051H      | Not Available |           | Positive        |
| Tranoroa                      |                 | 55       | NCTC 10252     | Not Available |           | Positive        |
| Typhi                         |                 | D1       | ATCC 6539      | Not Available |           | Positive        |
| Typhimurium                   |                 | B        | ATCC 14028     | Animal tissue |            | positive        |
| Utrecht                      |                 | 52       | NCTC 10077     | Not Available |           | Positive        |
| Urbana                        |                 | N        | UPenn STS 110  | Not Available |           | Positive        |
| Vellore                       |                 | B        | ATCC 15611     | Rectal swab  |            | Positive        |
| Virchow                       |                 | C1       | ATCC 51955     | Not Available |           | Positive        |
| Volta                         |                 | F        | QL024.9        | Raw material |            | Positive        |
| Westhampton                   |                 | E1       | QL024.14       | Dog kibble   |            | Positive        |
| Worthington                   |                 | G        | UPenn STS 114  | Not Available |           | Positive        |
| Zwickau                       |                 | I        | NCTC 15805     | Not Available |           | Positive        |
| **S. enterica subsp. enterica** |                 | 66       | ATCC 43975     | Not Available |           | Positive        |
| **S. enterica subsp. enterica** |                 | 66       | NCTC 12419     | Not Available |           | Positive        |

*S. enterica* subsp. *enterica* strains and their associated characteristics are listed. Each strain is identified by its subspecies, strain name, code, ATCC or other strain collection number, sample type, and availability status. The results indicate whether the strain is positive for the designated condition.

*ATCC — American Type Culture Collection*  
*QL — Q Laboratories Culture Collection*  
*NCTC — National Collection of Type Cultures*  
*FDA — U.S. Food and Drug Administration Culture Collection*  
*UPenn — University of Pennsylvania Culture Collection*
Table 2. Exclusivity Testing Results for the OBOP Salmonella Method

| Organism                          | Source and Strain No. | Origin                      | OBOP Salmonella Result                      |
|-----------------------------------|-----------------------|-----------------------------|---------------------------------------------|
| *Aeromonas hydrophila*            | ATCC 49140            | 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 alvi*                     | 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                        |
| Organism                        | ATCC       | Medium            | Result               |
|--------------------------------|------------|-------------------|----------------------|
| *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 |

*ATCC – American Type Culture Collection*
| Matrix          | Strain                      | Level (CFU/portion) | N | OBOP Salmonella Presumptive | OBOP Salmonella Confirmed |
|-----------------|-----------------------------|--------------------|----|-----------------------------|---------------------------|
|                 |                             | x                  | POD_{95} | 95% CI          | x | POD_{95} | 95% CI          | dPOD_{95} | 95% CI          |
| Chili powder    | *Salmonella Oranienberg* ATCC 9239 | 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 CDC' 147* | 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 Agona CDC 1201-82* | 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 |
|                 |                             | 6.0 (1.7, 22)      | 5   | 5 | 1   | 0.57, 1  | 5 | 1   | 0.57, 1  | 0 | -0.47, 0.47 |
| Queso Fresco    | *Salmonella Agona ATCC 51957* | 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 |
|                 |                             | 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 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 | *Salmonella Othmarschen 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 |
| Dry Pet Food, 375 g | *Salmonella Othmarschen 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 |
| Matrix                  | Strain          | Level (CFU/portion) | N | OBOP Salmonella Presumptive | OBOP Salmonella Confirmed |
|------------------------|-----------------|--------------------|---|-----------------------------|---------------------------|
|                        |                 |                    |   | x' | PODc | 95% CI | x | PODc | 95% CI | dPOD |
| Cantaloupe             | Salmonella      |                    | 5 | 0  | 0    | 0.43  | 0  | 0    | 0.43  | 0    |
|                        | Newport ATCC 6962 | 0.55 (0.29, 0.93)  | 20| 11 | 0.55 | 0.34, 0.74 | 11 | 0.55 | 0.34, 0.74 | 0    |
|                        |                 | 3.7 (1.0, 9.0)     | 5 | 5  | 1    | 0.57, 1 | 5  | 1    | 0.57, 1 | 0    |
| Smoked Salmon          | Salmonella      |                    | 5 | 0  | 0    | 0.43  | 0  | 0    | 0.43  | 0    |
|                        | Montevideo ATCC 8387 | 0.50 (0.25, 0.86)  | 20| 8  | 0.40 | 0.22, 0.61 | 8  | 0.40 | 0.22, 0.61 | 0    |
|                        |                 | 2.0 (0.91, 4.3)    | 5 | 5  | 1    | 0.57, 1 | 5  | 1    | 0.57, 1 | 0    |
| Raw Ground Turkey, 25 g | Salmonella      |                    | 5 | 0  | 0    | 0.43  | 0  | 0    | 0.43  | 0    |
|                        | Typhimurium ATCC 14028 | 1.0 (0.61, 1.7)    | 20| 14 | 0.70 | 0.48, 0.85 | 14 | 0.70 | 0.48, 0.85 | 0    |
|                        |                 | 2.6 (1.1, 5.8)    | 5 | 5  | 1    | 0.57, 1 | 5  | 1    | 0.57, 1 | 0    |
| Raw Ground Turkey, 325 g | Salmonella      |                    | 5 | 0  | 0    | 0.43  | 0  | 0    | 0.43  | 0    |
|                        | Typhimurium ATCC 14028 | 1.0 (0.61, 1.7)    | 20| 11 | 0.55 | 0.34, 0.74 | 11 | 0.55 | 0.34, 0.74 | 0    |
|                        |                 | 2.6 (1.1, 5.8)    | 5 | 5  | 1    | 0.57, 1 | 5  | 1    | 0.57, 1 | 0    |
| Pasteurized Liquid Egg | Salmonella      |                    | 5 | 0  | 0    | 0.43  | 0  | 0    | 0.43  | 0    |
|                        | Enteritidis ATCC 13076 | 0.35 (0.14, 0.62)  | 30| 9  | 0.30 | 0.17, 0.48 | 9  | 0.30 | 0.17, 0.48 | 0    |
|                        |                 | 43 (10, 186)       | 5 | 5  | 1    | 0.57, 1 | 5  | 1    | 0.57, 1 | 0    |
| Pasteurized Liquid Egg | Salmonella      |                    | 5 | 0  | 0    | 0.43  | 0  | 0    | 0.43  | 0    |
|                        | Enteritidis ATCC 13076 | 0.75 (0.44, 1.2)   | 20| 11 | 0.55 | 0.34, 0.74 | 11 | 0.55 | 0.34, 0.74 | 0    |
|                        |                 | 3.7 (1.0, 9.0)    | 5 | 5  | 1    | 0.57, 1 | 5  | 1    | 0.57, 1 | 0    |
| Chicken Carcass Rinse  | Salmonella      |                    | 5 | 0  | 0    | 0.43  | 0  | 0    | 0.43  | 0    |
|                        | Kentucky ATCC 9263 | 26 | 20 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0    |
|                        |                 | 54 | 5  | 5  | 1    | 0.57, 1 | 5  | 1    | 0.57, 1 | 0    |
| Matrix   | Strain                  | Level (CFU/portion) | N^b | x^c | POD_{cp}^d | 95% CI   | x | POD_{cc}^e | 95% CI   | dPOD_{cp}^f | 95% CI^g |
|----------|-------------------------|--------------------|-----|-----|------------|----------|---|------------|----------|-------------|----------|
| Stainless Steel^k | *Salmonella Derby* NCTC® 5721 + *Citrobacter freundii* ATCC 8090 | 59/720^o          | 20  | 8   | 0.40       | 0.22, 0.61 | 8 | 0.40       | 0.22, 0.61 | 0           | -0.13, 0.13 |
|          |                         | 240/3100^o         | 5   | 5   | 1          | 0.57, 1   | 5 | 1          | 0.57, 1   | 0           | -0.47, 0.47 |

^aFrom MPN analysis.
^bN = Number of test portions.
^cNumber of positive test portions.
^dPOD_{cp} = Candidate method presumptive positive outcomes divided by the total number of trials.
^ePOD_{cc} = Candidate method confirmed positive outcomes divided by the total number of trials.
^f\text{dPOD}_{cp} = \text{Difference between the candidate method presumptive result and candidate method confirmed result POD values.}
^g95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.
^hAmerican Type Culture Collection, Manassas, VA.
^iCenters for Disease Control and Prevention, Atlanta, GA.
^jTrial performed by the independent laboratory.
^kTrial performed by an independent third-party laboratory as part of the MicroVal study.
^lQ Laboratories, Cincinnati, OH.
^mCFU per carcass, determined by plate count of the inoculum culture.
^nNational Collection of Type Cultures, Salisbury, UK.
^oCFU per test area, determined by plate count of the inoculum culture.
Table 4. Method Comparison Results: OBOP Salmonella Confirmed vs. Reference Method

| Matrix                | Strain                          | Level (CFU/portion)\(^a\) | \(x^b\) | POD;\(^c\) | 95% CI    | \(x^b\) | POD;\(^c\) | 95% CI    | dPOD;\(^c\) | 95% CI\(^d\) |
|-----------------------|---------------------------------|---------------------------|---------|------------|-----------|---------|------------|-----------|------------|-------------|
| Chili powder          | *Salmonella* Oranienberg        | -                         | 5       | 0          | 0.43      | 0       | 0          | 0.43      | 0          | -0.43, 0.43 |
|                       | ATCC\(^b\) 9239                | 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          | 0.43      | 0       | 0          | 0.43      | 0          | -0.43, 0.43 |
|                       | CDC\(^c\) 147                  | 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* Agona              | -                         | 5       | 0          | 0.43      | 0       | 0          | 0.43      | 0          | -0.43, 0.43 |
|                       | CDC 1201-82                    | 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 |
|                       |                                 | 6.0 (1.7, 22)             | 5       | 5          | 1         | 0.57, 1   | 5       | 1          | 0.57, 1   | 0, -0.43, 0.43 |
| Queso Fresco\(^d\)   | *Salmonella* Agona              | -                         | 5       | 0          | 0.43      | 0       | 0          | 0.43      | 0          | -0.43, 0.43 |
|                       | ATCC 51957                     | 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 |
|                       |                                 | 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          | 0.43      | 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\(^e\) | *Salmonella* Othmarschen      | -                         | 5       | 0          | 0.43      | 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 |
| Dry Pet Food, 375 g\(^e\) | *Salmonella* Othmarschen      | -                         | 5       | 0          | 0.43      | 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 |
| Matrix                  | Strain                | Level (CFU/portion) | N° | OBOP Salmonella Confirmed | Reference Method |
|------------------------|-----------------------|--------------------|----|---------------------------|------------------|
|                        |                       |                    |    | x | PODc | 95% CI | x | PODc | 95% CI | dPODc | 95% CI |
| Cantaloupe             | Salmonella Newport ATCC 6962 | 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 |
| Smoked Salmon          | Salmonella Montevideo 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 |
| Raw Ground Turkey, 25 g | Salmonella Typhimurium 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 |
| Raw Ground Turkey, 325 g | Salmonella Typhimurium 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 |
| Pasteurized Liquid Egg | Salmonella Enteritidis 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 |
| Pasteurized Liquid Egg | Salmonella Enteritidis 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 |
| Chicken Carcass Rinse  | Salmonella Kentucky ATCC 9263 | 26                | 20 | 10 | 0.50 | 0.30, 0.70 | 6  | 0.30 | 0.15, 0.52 | 0.20 | -0.10, 0.45 |
|                        |                       | 54                | 5  | 1  | 0.57 | 0.57, 1   | 5  | 1   | 0.57, 1   | 0   | -0.43, 0.43 |
| Matrix          | Strain                  | Level (CFU/portion) | N<sup>x</sup> | POD<sub>C</sub> | 95% CI   | POD<sub>R</sub> | 95% CI   | dPOD<sub>C</sub> | 95% CI   |
|-----------------|-------------------------|--------------------|--------------|----------------|----------|----------------|----------|-----------------|----------|
| Stainless Steel | *Salmonella Derby* NCTC<sup>n</sup> 5721 + *Citrobacter freundii* ATCC 8090 | 59/720<sup>o</sup> | 20           | 8              | 0.40     | 0.22, 0.61     | 0.18, 0.57 | 0.05            | -0.23, 0.32 |
|                 |                         | 240/3100<sup>o</sup> | 5            | 5              | 1        | 0.57, 1       | 0.57, 1   | 0           | -0.43, 0.43   |

<sup>a</sup>From MPN analysis.

<sup>b</sup>N = Number of test portions.

<sup>c</sup>N = Number of positive test portions.

<sup>d</sup>POD<sub>C</sub> = Candidate method confirmed positive outcomes divided by the total number of trials.

<sup>e</sup>POD<sub>R</sub> = Reference method confirmed positive outcomes divided by the total number of trials.

<sup>f</sup>dPOD<sub>C</sub> = Difference between the candidate method and reference method POD values.

<sup>g</sup>95% CI = If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

<sup>h</sup>American Type Culture Collection, Manassas, VA.

<sup>i</sup>Centers for Disease Control and Prevention, Atlanta, GA.

<sup>j</sup>Trial performed by the independent laboratory.

<sup>k</sup>Trial performed by an independent third-party laboratory as part of the MicroVal study.

<sup>l</sup>Q Laboratories, Cincinnati, OH.

<sup>m</sup>CFU per carcass, determined by plate count of the inoculum culture.

<sup>n</sup>National Collection of Type Cultures, Salisbury, UK.

<sup>o</sup>CFU per test area, determined by plate count of the inoculum culture.
Table 5. Results of Robustness Testing for the OBOP *Salmonella* Method

| Condition | Enrichment Incubation Temperature (°C) | CASE Agar Incubation Time (h) | CASE Agar Incubation Temperature (°C) | % Positive Results$^a$ |
|-----------|---------------------------------------|-------------------------------|---------------------------------------|-----------------------|
| 1         | 40.5                                  | 21                            | 35                                    | 0                     | 50                    |
| 2         | 40.5                                  | 21                            | 39                                    | 30                    | 20                    |
| 3         | 42.5                                  | 21                            | 35                                    | 0                     | 10                    |
| 4         | 42.5                                  | 21                            | 39                                    | 10                    | 70                    |
| 5         | 40.5                                  | 27                            | 35                                    | 0                     | 60                    |
| 6         | 40.5                                  | 27                            | 39                                    | 0                     | 50                    |
| 7         | 42.5                                  | 27                            | 35                                    | 0                     | 20                    |
| 8         | 42.5                                  | 24                            | 37                                    | 0                     | 50                    |

$^a$10 replicates tested.

$^b$Enriched uninoculated queso fresco.

$^c$Queso fresco inoculated with *Salmonella* enterica ser. Agona at ~0.5-1.0 CFU/test portion.

$^d$Standard conditions for the OBOP *Salmonella* method.