Comparative Evaluation of the 3M™ Petrifilm™ *Salmonella* Express System for the Detection of *Salmonella* species in Food and Environmental Surfaces

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A validation study of the 3M™ Petrifilm™ *Salmonella* Express (SALX) System for the detection and biochemical confirmation of *Salmonella* species was conducted at Q Laboratories, Inc., Cincinnati, OH. The 3M Petrifilm SALX System was compared to the USDA/FSIS-MLG 4.05 (2011) *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg and Catfish Products* for raw ground chicken, pasteurized liquid whole eggs, raw ground beef, raw ground pork and cooked chicken nuggets, to the FDA/BAM Chapter 5 *Salmonella* method for frozen uncooked shrimp, fresh spinach, dry dog food and stainless steel environmental surfaces and to the ISO 6579: *Microbiology of food and animal feeding stuffs—Horizontal method for the detection of Salmonella spp.* for raw ground chicken and pasteurized liquid whole eggs. Twenty replicates of each matrix were analyzed at one inoculum level: 0.2–2 CFU/test portion. Five control replicates were also analyzed at 0 CFU/test portion (un-inoculated). Results were analyzed by the Mantel-Haenszel chi-square analysis for unpaired test portions. There was no statistically significant difference in the number of positive samples detected by the 3M Petrifilm SALX System and the reference methods for all nine matrices analyzed. The 3M Petrifilm SALX System demonstrated reliability as an alternative qualitative identification method for the detection and biochemical confirmation of *Salmonella* in select foods and environmental surface.

This report presents the analytical results for comparison of the 3M™ Petrifilm™ *Salmonella* Express (SALX) System to the USDA/FSIS MLG 4.05 (2011) *Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Eggs and Catfish Products*, the FDA/BAM Chapter 5 *Salmonella* and the ISO 6579: *Microbiology of food and animal feeding stuffs—Horizontal method for the detection of Salmonella spp.* reference methods. All analyses were conducted at Q Laboratories, Inc. (Cincinnati, OH). All test kits and proprietary media unique to the 3M Petrifilm SALX System were provided by 3M Food Safety Department (St. Paul, MN).

Materials and Methods

The methodology for this study was followed as outlined in the AOAC® Research Institute *Performance Tested Method℠* Program Protocol: Validation Outline for 3M™ Petrifilm™ *Salmonella* Express System. The system consists of 3M™ *Salmonella* Enrichment Base, 3M™ *Salmonella* Enrichment Supplement, 3M™ Petrifilm™ *Salmonella* Express Plate and the 3M™ Petrifilm™ *Salmonella* Express Confirmation Disk. The study consisted of evaluating a total of 25 samples for each of eight food matrices and one environmental surface. Within each sample set there were five un-inoculated samples and 20 low-level inoculated samples. The target levels of each strain of *Salmonella* used for challenging each matrix were as follows: 0.2–2 colony forming units (CFU)/test portion for the low level inoculation and 0 CFU/test portion for the un-inoculated control samples.

Each matrix was inoculated with a different strain of *Salmonella* species as indicated in Table A below. A diluted 24 hour broth culture inoculum was added to a bulk sample of each food matrix and mixed thoroughly. The cooked chicken nuggets and pasteurized liquid whole eggs were inoculated with an organism that had been heat stressed for ten minutes at 50°C in a water bath. The degree of injury of the culture was estimated by plating an aliquot of diluted culture onto Xylose Lysine Desoxycholate (XLD) and Tryptic Soy agar (TSA). The agars were incubated at 37 ± 1°C for 24 ± 2 hours and the colonies were counted. The degree of injury was estimated as $(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$, where n_{select} = number of colonies on selective agar and $n_{nonselect}$ = number of colonies on nonselective agar. Results of the percent injury are presented in Table 1 of the Appendix. Following inoculation, all test portions were mixed thoroughly and were held at 2–5°C for 48–72 hours prior to analysis to allow time for the organisms to equilibrate within the sample. For the analysis of the dry dog food test portions, a bulk lot of test product was inoculated with a lyophilized culture and held at 25 ± 2°C for two weeks to allow time for the organism to equilibrate. Test portions analyzed by the 3M Petrifilm SALX System were prepared by mixing 25g of inoculated test product with 350g of un-inoculated test product. 25g of inoculated test product were analyzed by the FDA/BAM method. For the analysis of the cooked chicken nuggets, 25g of inoculated test product was mixed with 300g of un-inoculated test product and analyzed by both the candidate and reference method. Fractionally positive results, those in which ether the reference or candidate method yields 5–15 positive results out of the 20 low level inoculum replicates, were required for each matrix.

Table A: Test Matrices and Inoculating Organisms

Matrix	Inoculating Organism
Raw Ground Chicken	*Salmonella enterica* spp. Heidelberg NCTC 5717
Pasteurized Liquid Whole Eggs	*Salmonella enterica* spp. Enteritidis ATCC 13076
Dry Dog Food	*Salmonella enterica* spp. Poona NCTC 4840
Raw Ground Pork	*Salmonella enterica* spp. Montevideo ATCC 8387
Stainless Steel Environmental Surface	*Salmonella enterica* spp. Kahla ATCC 17980
Frozen, Uncooked Shrimp	*Salmonella enterica* spp. Virchow ATCC 51955
Fresh Spinach	*Salmonella enterica* spp. Saint Paul ATCC 9712
Raw Ground Beef	*Salmonella enterica* spp. Ohio STS 81*
Cooked Chicken Nuggets	*Salmonella enterica* spp. Typhimurium ATCC 14028

*Culture obtained from the University of Pennsylvania culture collection.

Environmental surfaces were inoculated at a level expected to yield fractional recovery. Replicate 4" x 4" stainless steel surfaces areas were inoculated with 0.10mL of diluted culture and dried for 16–24 hours at room temperature (24 ± 2°C). The inoculation level for the stainless steel test portions was determined by plating the inoculum onto Tryptic Soy agar (TSA) in triplicate. Sampling sponges, pre-moistened with Dey-Engley neutralizing broth, were used to sample each test area using horizontal and vertical motions. The sponges were held at room temperature for at least two hours prior to analysis.

A background screen of each food matrix was conducted prior to inoculation and no natural contamination of the target organisms were detected in the test matrices.

The level of *Salmonella* was determined by Most Probable Number (MPN) on the day of analysis by analyzing 3 x 100g, 20 x 25g (reference method test portions) and 3 x 5g inoculated test samples for the raw ground chicken, raw ground beef, raw ground pork, frozen uncooked shrimp and fresh spinach. For the cooked chicken nuggets and dry dog food, 5 x 75g, 5 x 25g (from the bulk lot of inoculated test product) and 5 x 10g test portions were analyzed. For the pasteurized liquid whole eggs, 5 x 200g, the 20 reference method test portions and 5 x 25g test portions were analyzed. Each test portion was enriched with the appropriate reference method enrichment broth at a 1:10 dilution and analyzed by the reference method procedure. The number of positives from the three test levels was used to calculate the MPN using the AOAC® MPN calculator (http://www.lcfltd.com/customer/LCFMPNCalculator.exe).

Detailed MPN results for each matrix are provided in Table 2A through 2C of the Appendix.

3M Petrifilm *Salmonella* Express System Method Comparison

USDA/FSIS-MLG 4.05 Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg and Catfish

For the USDA/FSIS method, all test portions for each food product, raw ground chicken, pasteurized liquid whole eggs, raw ground beef, raw ground pork and cooked chicken nuggets were prepared as outlined in the study protocol. Following equilibration of the microorganism in the matrix, 25 test portions consisting of 25g for raw ground chicken, raw ground beef and raw ground pork were enriched with 225mL of Buffered Peptone Water (BPW) and homogenized for two minutes. For cooked chicken nuggets, 25 test portions consisting of 325g were enriched with 1500mL of BPW and homogenized for two minutes. After mixing, an additional 1425mL of BPW was added to the sample. For pasteurized liquid whole eggs, 25 test portions of 100g were enriched with 900mL of BPW and homogenized for two minutes. All test portions were incubated at 35 ± 2°C for 20–24 hours.

After incubation, 0.10mL of primary enrichment for each sample was transferred to 10mL of modified Rappaport-Vassiliadis broth (mRV) and 0.50–10mL of Tetrathionate Hanja (TTH) broth. The mRV and TTH tubes were incubated at 42 ± 0.5°C for 22–24 hours. Following incubation, a loopful of each secondary enrichment was streaked to Brilliant Green Sulfa (BGS) agar and Xylose Lysine Tergitol (XLT4) agar and incubated at 35 ± 2°C for 18–24 hours. If no visible colonies were present, both the BGS and XLT4 plates were re-incubated for an additional 18–24 hours at 35 ± 2°C. Up to three suspect colonies from each selective agar were transferred to Triple Sugar Iron agar (TSI) and Lysine Iron agar (LIA) and incubated at 35 ± 2°C for 22–26 hours. Growth from samples producing typical biochemical reactions in TSI and LIA, were streaked to TSA slants and incubated for 18–24 hours at 35 ± 2°C. Growth from the TSA slant was used to conduct the flagellar H serological test and polyvalent somatic O serological test and for biochemical confirmation. Growth from the TSA slants was used for final confirmation of *Salmonella* by VITEK® 2 GN following AOAC® *Official Methods of Analysis℠* 2011.17.

FDA/BAM Chapter 5 *Salmonella*

For the FDA/BAM method, all test portions for each food product, frozen uncooked shrimp, fresh spinach, dry dog food and stainless steel environmental surfaces were prepared as outlined in the study protocol. Following equilibration of the microorganism in the matrix, 25 test portions consisting of 25g for each food matrix were enriched with 225mL of Lactose broth and homogenized for two minutes. For sponges used to analyze the stainless steel environmental surfaces, each sponge was enriched with 225mL of Lactose broth and mixed by hand massaging. All test portions were allowed to stand for 60 minutes to adjust pH to 6.8 ± 0.2 if necessary. Test portions for each matrix were incubated for 22–26 hours at 35 ± 2°C.

After incubation of the samples, a 0.10mL aliquot of the primary enrichment for each sample was transferred to 10mL of Rappaport-Vassiliadis medium (RV) and a 1mL aliquot was transferred to 10mL of Tetrathionate (TT) broth. RV tubes were incubated for 22–26 hours at 42 ± 0.2°C. For high microbial load foods (fresh spinach and frozen uncooked shrimp), TT tubes were incubated for 22–26 hours at 43 ± 0.2°C in a circulating water bath. For low microbial load foods (dry dog food and stainless steel environmental surfaces), TT tubes were incubated 22–26 hours at 35 ± 2°C. Following incubation, a loopful of each secondary enrichment was streaked to Bismuth Sulfite (BS) agar, Hektoen Enteric (HE) agar and XLD agar and incubated at 35 ± 2°C for 22–26 hours. If no visible colonies were present after 24 hours of incubation, BS plates were re-incubated for an additional 22–26 hours at 35 ± 2°C. Up to two or more suspect colonies from each selective agar were transferred to TSI and LIA and incubated at 35 ± 2°C for 22–26 hours. Growth from samples producing typical biochemical reactions in TSI and LIA, were streaked to TSA slants and incubated for 18–24 hours at 35 ± 2°C. Growth from the TSA slant was used to conduct the flagellar H serological test and polyvalent and individual somatic O serological test and for biochemical confirmation. Growth from the TSA slants was used for final confirmation of *Salmonella* by VITEK® 2 GN following AOAC® *Official Methods of Analysis℠* 2011.17.

ISO 6579: Microbiology of food and animal feeding stuffs—Horizontal method for the detection of *Salmonella* spp.

For the ISO 6579 method, all test portions were for each food product, raw ground chicken and pasteurized liquid whole egg were prepared as outlined in the study protocol. Following equilibration of the microorganism in the matrix, 25 test portions consisting of 25g for each matrix were enriched with 225mL of BPW. Samples were homogenized for two minutes. Samples were incubated at 37 ± 1°C for 18 ± 2 hours. After incubation, a 0.10mL aliquot of the primary enrichment for each sample was transferred to a 10mL tube of modified Rappaport-Vassiliadis broth with soya (RVS) and a 1mL aliquot added to 10mL of Muller-Kauffmann Tetrathionate-novobiocin broth (MKTTn). The RVS was incubated for 21–27 hours at 41.5 ± 1°C. The MKTTn tubes were incubated for 21–27 hours at 37 ± 1°C. A loop full each of RVS and MKTTn were streaked onto XLD and HE selective agars and incubated at 37 ± 1°C for 24 ± 3 hours. Typical colonies were streaked onto Trypticase Soy agar (TSA) and Semi-Solid Nutrient Agar (SSNA) and incubated for 24 ± 3 hours at 37 ± 1°C. Growth from TSA was used to conduct the somatic O serological test. Growth from SSNA was used to conduct flagellar H serological test and for biochemical confirmation. Growth from the TSA slants was used for final confirmation of *Salmonella* by VITEK® 2 GN following AOAC® *Official Methods of Analysis℠* 2011.17.

3M Petrifilm *Salmonella* Express (SALX) System

After equilibration of the inoculum, 25 test portions for each matrix were enriched using pre-warmed (41.5 ± 1°C) 3M *Salmonella* Enrichment Base containing 3M *Salmonella* Enrichment Supplement. For the analysis of raw ground beef, raw ground pork, frozen uncooked shrimp and fresh spinach, 25g test portions were enriched in 225mL of 3M *Salmonella* Enrichment Base. For the analysis of cooked chicken nuggets, 325g test portions were enriched with 2925mL of 3M *Salmonella* Enrichment Base. For the analysis of pasteurized liquid whole eggs, 100g test portions were enriched with 900mL of 3M *Salmonella* Enrichment Base. For the analysis of dry dog food, 375g samples were enriched with 3375mL of 3M *Salmonella* Enrichment Base. Sponges used to sample stainless steel environmental surfaces were enriched with 225mL of 3M *Salmonella* Enrichment Base. All matrices were homogenized for two minutes and incubated at 41.5 ± 1°C for 18–24 hours.

Prior to analysis, the 3M Petrifilm SALX Plates were hydrated using 2mL of sterile distilled water. After hydration, the liquid was spread across of the surface of the plate using a plastic spreader to evenly distribute the diluent. The plates were left undisturbed and protected from light for a minimum of one hour prior to use. For foods with low microbial loads (pasteurized liquid whole egg, dry dog food, cooked chicken nuggets and stainless steel environmental surfaces), test portions were streaked in duplicate after 18 and 24 hours of primary enrichment. For foods that contained a high microbial load (raw ground chicken, frozen uncooked shrimp, fresh spinach, raw ground beef and raw ground pork), a 0.10mL aliquot of the primary enrichment was transferred into 10mL of Rappaport-Vassiliadis R10 (RV [R10]) broth at both 18 and 24 hours of primary enrichment and incubated at 41.5 ± 1°C for 8 and 24 hours. At 8 and 24 hours, a loopful of each test portion was streaked in duplicate to 3M Petrifilm SALX Plates and the plates were incubated at 41.5 ± 1°C for 24 ± 2 hours. Test portions from both low and high microbial foods were confirmed following two procedures—traditional confirmation using secondary selective enrichments and an alternative confirmation directly from the 3M Petrifilm SALX Plates.

Alternative Confirmation

After incubation of the 3M Petrifilm SALX Plates, plates were observed for growth of presumptive *Salmonella* colonies, red to brown colonies with discrete yellow zones and/or gas bubbles. Using a fine tip marker, presumptive positive colonies were circled on the top of the plate's film. The film was lifted and a 3M Petrifilm *Salmonella* Express Confirmation Disk was placed onto the gel. The film was closed and, using a gloved hand (while practicing good laboratory technique), air bubbles were removed by gently applying a sweeping motion with even pressure onto the top of the film with the analyst's fingers. The plates with confirmation disks were incubated at 41.5 ± 1°C for 4–5 hours. After incubation, the circled presumptive colonies were observed. A color change from red/brown to green blue, blue, dark blue or black confirmed the colony as *Salmonella* species. If the circled colony remained red or brown it was marked as negative. Typical colonies were picked to TSI and LIA and samples were confirmed following traditional biochemical tests for *Salmonella* species by each reference method including somatic O and flagellar H tests. Final confirmation was achieved by VITEK® 2 GN conducted per AOAC® *Official Methods of Analysis℠* 2011.17.

Traditional Confirmation

Aliquots from the primary enrichment of each test portion were also transferred to the secondary selective enrichments specified by each reference method, TT and RV for samples confirmed following the FDA/BAM method and TTH and RVS for samples confirmed following the USDA/FSIS-MLG method. After incubation, test portions from the secondary selective enrichments were streaked to selective agars specified by each reference method and positive samples were confirmed following traditional biochemical tests for *Salmonella* species by each reference method including somatic O and flagellar H tests. Final confirmation at each time point was achieved by VITEK® 2 GN conducted per AOAC® *Official Methods of Analysis℠* 2011.17.

Results and Discussion

For the method comparison, results obtained from samples analyzed by the 3M Petrifilm SALX System were comparable to those analyzed by the FDA/BAM Chapter 5, USDA/FSIS-MLG 4.05 and ISO 6579 reference methods. A Mantel-Haenszel chi-square analysis ($\chi 2$) for unmatched test portions between the 3M Petrifilm SALX System samples and the reference method samples indicated that there was no statistically significant difference between the number of positive results given by the two methods being compared for all nine matrices. A summary of the results is presented in Tables B and C on Page 9. Detailed results of the chi-square analysis are provided in Tables 3–13 of the Appendix.

Based on the results obtained from the MPN analyses displayed in Tables 2A and 2B of the Appendix, the target levels of 0.2–2 CFU/test portion for the low inoculum level were achieved for each matrix tested. In addition, the requirement of obtaining fractionally positive results (5–15 positives out of 20 replicates) was achieved by either the candidate or reference methods or both for each of the matrices analyzed in this study.

High Microbial Foods

A. Raw Ground Chicken

For raw ground chicken test portions, there were fifteen confirmed positives for the 3M Petrifilm SALX System at all four time points (18 hours and 24 hours of primary enrichment transferred into RV[R10] secondary enrichment and streaked at 8 and 24 hours) and fourteen confirmed positives for the USDA/FSIS-MLG and ISO 6579 methods. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.12 was obtained between the reference methods and the candidate method when using the alternative confirmation indicating no significant difference between the candidate and either reference method at all four time points. A $\chi 2$ value of 0.00 was obtained between the reference methods and the candidate method when using the traditional confirmation, indicating no significant difference between the candidate and either reference methods at any of the time points. Detailed results are presented in Table 3 of the Appendix.

B. Frozen Uncooked Shrimp

For frozen uncooked shrimp test portions, there were twelve confirmed positives for the 3M Petrifilm SALX System at all four time points and ten confirmed positives for the FDA/BAM method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.39 was obtained between the reference and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the time points. Detailed results are presented in Table 7 of the Appendix.

C. Fresh Spinach

For fresh spinach test portions, there were eleven confirmed positives for the 3M Petrifilm SALX System for both 18 and 24 hour primary enrichment samples transferred to RV-R10 and incubated for 24 hours and confirmed following both the traditional and alternative confirmation procedures. For test portions enriched for 18 and 24 hours and transferred to RV-R10 and incubated for eight hours there were seven confirmed positives by the alternative confirmation and eleven confirmed positives by the traditional confirmation indicating the presence of four false negative results. There were ten confirmed positives for samples analyzed by the FDA/BAM method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.10 was obtained between the reference method and the candidate method using the traditional confirmation for all four time points. For candidate samples confirmed following the alternative confirmation procedure, a $\chi 2$ value of 0.10 was obtained for samples enriched for either 18 hours or 24 hours in the primary enrichment, transferred to RV-R10 and incubated for 24 hours. For candidate samples confirmed following the alternative confirmation procedure, a $\chi 2$ value of 0.90 was obtained for samples enriched for either 18 hours or 24 hours in the primary enrichment, transferred to RV-R10 and incubated for 8 hours. The chi-square values indicate no significant difference between the candidate and reference method at any of the time points. Detailed results are presented in Table 8 of the Appendix.

D. Raw Ground Beef

For raw ground beef test portions, there were thirteen confirmed positives for the 3M Petrifilm SALX System at all four time points and thirteen confirmed positives for the USDA/FSIS-MLG method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.00 was obtained between the reference and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the four time points. Detailed results are presented in Table 11 of the Appendix.

E. Raw Ground Pork

For raw ground pork test portions, there were nine confirmed positives for the 3M Petrifilm SALX System at all four time points and nine confirmed positives for the USDA/FSIS-MLG method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.00 was obtained between the reference and candidate method samples using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the four time points. Detailed results are presented in Table 12 of the Appendix.

Low Microbial Foods

A. Pasteurized Liquid Whole Eggs

For pasteurized liquid whole egg test portions, there were six confirmed positives for the 3M Petrifilm SALX System at both time points and four confirmed positives for the USDA/FSIS-MLG method and five confirmed positives for the ISO 6579 method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.98 was obtained between the USDA/FSIS-MLG and candidate method using both traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference method at any of the time points. A $\chi 2$ value of 0.12 was obtained between the ISO 6579 and candidate method using both traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at both the 18 hour and 24 hour primary enrichment time points. Detailed results are presented in Tables 5 and 6 of the Appendix.

B. Dry Dog Food

For dry dog food test portions, there were sixteen confirmed positives for the 3M Petrifilm SALX System at both the 18 hour and 24 hour primary enrichment time points and fourteen confirmed positives for the FDA/BAM method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.52 was obtained between the reference and candidate method using both the alternative and traditional confirmation procedures, indicating no significant difference between the candidate and reference methods at either of the time points. Detailed results are presented in Table 9 of the Appendix.

C. Stainless Steel Environmental Surface

For stainless steel environmental surfaces, there were six confirmed positives for the 3M Petrifilm SALX System at both the 18 hour and 24 hour primary enrichment time points and seven confirmed positives for the FDA/BAM method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.11 was obtained between the reference and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the time points. Detailed results are presented in Table 10 of the Appendix.

D. Cooked Chicken Nuggets

For cooked chicken nugget test portions, there were six confirmed positives for the 3M Petrifilm SALX System at both the 18 hour and 24 hour primary enrichment time points and six confirmed positives for the USDA/FSIS-MLG method. All un-inoculated control samples were negative for both methods. A $\chi 2$ value of 0.00 was obtained between the reference and candidate method using both the traditional and alternative confirmation procedures, indicating no significant difference between the candidate and reference methods at any of the time points. Detailed results are presented in Table 13 of the Appendix.

Observations

The results of this study demonstrate the reliability of the 3M Petrifilm *Salmonella* (SALX) System when compared to the traditional reference methods for the detection and biochemical confirmation of *Salmonella*. The 3M Petrifilm SALX System offers a significant savings by producing presumptive results in as little as 40 hours and biochemical confirmation in 44 hours. For foods with low microbial loads, the 3M Petrifilm SALX System reduced the need to transfer enriched test portions into secondary enrichments, thus reducing the time to confirmation by 24 hours. The 3M Petrifilm SALX Plates produced isolated colonies even when samples contained high microbial flora. Use of the 3M Petrifilm SALX Confirmatory Disk offers the benefit of producing a visual interpretation of the entire 3M Petrifilm SALX Plate as well as providing accurate results in the identification of typical colonies as confirmed positive *Salmonella* isolates. Each isolate that was identified as positive after placement of the 3M Petrifilm SALX Confirmatory Disk was confirmed biochemically as *Salmonella* spp. The 3M Petrifilm SALX System offers the benefits of rapid results, high sensitivity and high specificity.

During this evaluation, the 3M Petrifilm SALX System was evaluated for its ability to identify *Salmonella* isolates on the 3M Petrifilm SALX Plate prior to and after the use of the 3M Petrifilm SALX Confirmatory Disk. From three matrices with high microbial loads (raw ground chicken, fresh spinach and frozen, uncooked shrimp), a total of 111 typical isolated colonies on 3M Petrifilm SALX Plates were picked for biochemical confirmation prior to the addition of the 3M Petrifilm SALX Confirmation Disk. All 111 colonies confirmed biochemically as positive for *Salmonella* spp. Additionally, 111 colonies that had been determined as positive *Salmonella* colonies after placement of the 3M Petrifilm SALX Confirmation Disk onto the 3M Petrifilm SALX Plate were picked for biochemical confirmation. All 111 colonies confirmed biochemically as positive for *Salmonella* spp. No false positive or false negative results were obtained in the pre and post disking evaluation. The results of the biochemical identification of typical colonies prior to and after placement of the 3M Petrifilm SALX Confirmation Disk are presented in Table D.

For this method comparison, a Mantel Haenszel chi-square statistical analysis was conducted to determine if any significant differences were observed between the number of positive samples detected at the different enrichment time points. For low microbial foods, no significant differences were observed between the results obtained using the 3M Petrifilm SALX System after 18 hours or 24 hours of primary enrichment. A Mantel Haenszel chi-square statistical analysis for high microbial foods, except spinach, indicated that no significant difference was observed between the results obtained at all four time points (18 hour primary enrichment + 8 hour RVR10 enrichment, 18 hour primary enrichment + 24 hour RVR10 enrichment, 24 hour primary enrichment + 8 hour RVR10 enrichment and 24 hour primary enrichment + 24 hour RVR10 enrichment) when analyzed with the 3M Petrifilm SALX System. For spinach, no significant differences were observed between results of the 18 and 24 hour primary enrichment when the RVR10 enrichment was incubated for a full 24 hours.

Table B: Summary of Results for High Microbial Foods

Food	Method		3M Petrifilm SALX System								
			Presumptive				Confirmed (Alternative Confirmation)				Traditional Confirmation
			18 Hr Primary Enrichment		24 Hr Primary Enrichment		18 Hr Primary Enrichment		24 Hr Primary Enrichment		
			8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	8 Hr RV-R10	24 Hr RV-R10	
Raw Ground Chicken	USDA/FSIS-MLG	ISO 6579	15	15	15	15	15	15	15	15	14
	14	14									
Frozen Uncooked Shrimp	FDA/BAM		12	12	12	12	12	12	12	12	12
	10										
Fresh Spinach	FDA/BAM		7	11	7	11	7	11	7	11	11
	10										
Raw Ground Beef	USDA/FSIS-MLG		13	13	13	13	13	13	13	13	13
	13										
Raw Ground Pork	USDA/FSIS-MLG		9	9	9	9	9	9	9	9	9
	9										

Table C: Summary of Results for Low Microbial Foods

Food	Method		3M Petrifilm SALX System				
			Presumptive		Confirmed (Alternative Confirmation)		Traditional Confirmation
			18 Hr Primary Enrichment	24 Hr Primary Enrichment	18 Hr Primary Enrichment	24 Hr Primary Enrichment	
Pasteurized Liquid Whole Eggs	USDA/FSIS-MLG	ISO 6579	6	6	6	6	6
	4	5					
Dry Dog Food	FDA/BAM		16	16	16	16	16
	14						
Stainless Steel	FDA/BAM		6	6	6	6	6
	7						
Cooked Chicken Nuggets	USDA/FSIS-MLG		6	6	6	6	6
	6						

Table D: Summary of Results of Confirming Isolates Prior to and After Disking with the 3M Petrifilm SALX Confirmation Disk

Food	Number of Colonies Picked Prior to Disking				Number of Colonies Picked After Disking			
	Presumptive	Biochemically Confirmed	False Positive Rate	False Negative Rate	Presumptive	Biochemically Confirmed	False Positive Rate	False Negative Rate
Raw Ground Chicken	75	75	0%	0%	75	75	0%	0%
Frozen Uncooked Shrimp	60	60	0%	0%	60	60	0%	0%
Fresh Spinach	55	55	0%	0%	55	55	0%	0%

APPENDIX

Table 1: Percent Injury of Heat Stressed Organisms

Test Matrix	Test Organism	CFU/XLD	CFU/TSA	Percent Injury
Pasteurized Liquid Whole Eggs	*Salmonella enterica* sbsp. *enterica* serovar Enteritidis	4.4×10^7	1.1×10^8	60.0
Cooked Chicken Nuggets	*Salmonella enterica* sbsp. *enterica* serovar Typhimurium	6.0×10^7	1.3×10^8	53.8

$(1 - \frac{n_{select}}{n_{nonselect}}) \times 100$ Sample Calculation: $(1 - 5.2 \times 10^8/1.2 \times 10^9) \times 100 = 57\%$

Table 2A: MPN Summary Table

Test Matrix	CFU/4" x 4" (100cm²)
Stainless Steel Environment Surfaces	51

Table 2B: MPN Summary Table*

Low Inoculum Level	Raw Ground Chicken *S. enterica* ser. Heidelberg NCTC 5717			Frozen Uncooked Shrimp *S. enterica* ser. Virchow ATCC 51955			Fresh Spinach *S. enterica* ser. Saint Paul ATCC 9712			Raw Ground Beef *S. enterica* ser. Ohio STS 81			Raw Ground Pork *S. enterica* ser. Montevideo ATCC 8387		
(0.2–2 CFU/Test portion)	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
100g	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25g (20 Reference Samples)	14/20			10/20			10/20			13/20			9/20		
5g	–	–	–	+	–	–	–	+	–	–	+	–	–	–	+
MPN/test portion	1.15			0.77			0.77			1.07			0.66		
Low Conf. Limit/Test portion	0.70			0.41			0.41			0.65			0.38		
High Conf. Limit/Test portion	2.07			1.36			1.36			1.79			1.16		

*MPN was calculated using three 100g, three 5g and the 20 reference method samples and the AOAC® LCF MPN Calculator http://www.lcfltd.com/customer/LCFMPNCalculator.exe

Table 2C: MPN Summary Table*

Low Inoculum Level	Dry Dog Food *S. enterica* ser. Poona ATCC 4840[1]					Cooked Chicken Nuggets *S. enterica* ser. Typhimurium ATCC 14028[1]					Pasteurized Liquid Whole Eggs *S. enterica* ser. Enteritidis ATCC 13076[2]				
(0.2–2 CFU/Test Portion)	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
75g[1] or 200g[2]	+	+	–	–	+	+	+	+	+	–	+	–	+	+	+
25g (Inoculated Test Product)[1] or (20 Reference Samples)[2]	–	+	+	–	–	–	+	–	–	–	4/20				
10g[1] or 25g[2]	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
MPN/Test portion	0.03					0.03					0.32				
Low Conf. Limit/Test Portion	0.01					0.01					0.14				
High Conf. Limit/Test Portion	0.68					0.08					0.56				

*MPN was calculated using three 100g, three 5g and the 20 reference method samples and the AOAC® LCF MPN Calculator
[1]MPN was calculated for dry dog food and cooked chicken nuggets using five 75g, five 25g and five 10g samples and the AOAC® LCF MPN Calculator
[2]MPN was calculated for pasteurized liquid whole eggs using five 200g, five 25g and the 20 reference method samples and the AOAC® LCF MPN Calculator
http://www.lcfltd.com/customer/LCFMPNCalculator.exe

Table 3: 3M Petrifilm SALX System Summary Data Table for Raw Ground Chicken vs. USDA/FSIS-MLG

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX System Method			USDA/ FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0
18 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0
24 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0
24 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0

*Includes 95% confidence intervals

Table 4: 3M Petrifilm SALX System Summary Data Table for Raw Ground Chicken vs. ISO 6579

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX System Method			ISO 6579	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0
18 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0
24 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0
24 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	7.1×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Heidelberg NCTC 5717**		1.15 (0.70–2.07)	20	15	15	14	14	0.12	0.0

*Includes 95% confidence intervals

Table 5: 3M Petrifilm SALX System Summary Data Table for Pasteurized Whole Liquid Eggs vs. USDA/FSIS-MLG

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/100g*	Total Samples	3M Petrifilm SALX System Method			USDA/ FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment										
Control	N/A	6.0×10^1	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Enteritidis ATCC 13076**		1.15 (0.70–2.07)	20	6	6	6	4	0.98	0.98
24 Hr Primary Enrichment										
Control	N/A	6.0×10^1	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Enteritidis ATCC 13076**		1.15 (0.70–2.07)	20	6	6	6	4	0.98	0.98

*Includes 95% confidence intervals

Table 6: 3M Petrifilm SALX System Summary Data Table for Pasteurized Whole Liquid Eggs vs. ISO 6579

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/100g*	Total Samples	3M Petrifilm SALX System Method			ISO 6579	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment										
Control	N/A	6.0×10^1	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Enteritidis ATCC 13076**		1.15 (0.70–2.07)	20	6	6	6	5	0.12	0.12
24 Hr Primary Enrichment										
Control	N/A	6.0×10^1	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Enteritidis ATCC 13076**		1.15 (0.70–2.07)	20	6	6	6	5	0.12	0.12

*Includes 95% confidence intervals

Table 7: 3M Petrifilm SALX System Summary Data Table for Frozen Uncooked Shrimp vs. FDA/BAM

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX System Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41–1.36)	20	12	12	12	10	0.39	0.39
18 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41–1.36)	20	12	12	12	10	0.39	0.39
24 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41–1.36)	20	12	12	12	10	0.39	0.39
24 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	4.0×10^4	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Virchow ATCC 51955**		0.77 (0.41–1.36)	20	12	12	12	10	0.39	0.39

*Includes 95% confidence intervals

Table 8: 3M Petrifilm SALX System Summary Data Table for Fresh Spinach vs. FDA/BAM

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX System Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Saint Paul ATCC 9712**		0.77 (0.41–1.36)	20	7	7	11	10	0.90	0.10
18 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Saint Paul ATCC 9712**		0.77 (0.41–1.36)	20	11	11	11	10	0.10	0.10
24 Hr Primary Enrichment—8 Hr RV-R10 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Saint Paul ATCC 9712**		0.77 (0.41–1.36)	20	7	7	11	10	0.90	0.10
24 Hr Primary Enrichment—24 Hr RV-R10 Enrichment										
Control	N/A	1.0×10^7	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Saint Paul ATCC 9712**		0.77 (0.41–1.36)	20	11	11	11	10	0.10	0.10

*Includes 95% confidence intervals

Table 9: 3M Petrifilm SALX System Summary Data Table for Dry Dog Food vs. FDA/BAM

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/325g*	Total Samples	3M Petrifilm SALX System Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment										
Control	N/A	1.7×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Poona ATCC 4840**		0.03 (0.01–0.68)	20	16	16	16	14	0.52	0.52
24 Hr Primary Enrichment										
Control	N/A	1.7×10^3	< 0.075	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Poona ATCC 4840**		0.03 (0.01–0.68)	20	16	16	16	14	0.52	0.52

*Includes 95% confidence intervals

Table 10: 3M Petrifilm SALX System Summary Data Table for Stainless Steel Environmental Samples vs. FDA/BAM

Inoculation Level	Inoculating Organism	APC CFU/g	CFU/100cm²*	Total Samples	3M Petrifilm SALX System Method			FDA/BAM	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed				
						Alternative	Traditional			
18 Hr Primary Enrichment										
Control	N/A	N/A	0	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Kahla ATCC 17980**		51	20	6	6	6	7	0.11	0.11
24 Hr Primary Enrichment										
Control	N/A	N/A	0	5	0	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Kahla ATCC 17980**		51	20	6	6	6	7	0.11	0.11

*Includes 95% confidence intervals

Table 11: 3M Petrifilm SALX System Summary Data Table for Raw Ground Beef vs. USDA/FSIS-MLG

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX System Method		USDA/ FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed			
18 Hr Primary Enrichment—8 Hr RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65–1.79)	20	13	13	13	0.0	0.0
18 Hr Primary Enrichment—24 Hr RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65–1.79)	20	13	13	13	0.0	0.0
24 Hr Primary Enrichment—8 Hr RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65–1.79)	20	13	13	13	0.0	0.0
24 Hr Primary Enrichment—24 Hr RV-R10 Enrichment									
Control	N/A	3.4×10^6	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Ohio STS 81**		1.07 (0.65–1.79)	20	13	13	13	0.0	0.0

*Includes 95% confidence intervals

Table 12: 3M Petrifilm SALX System Summary Data Table for Raw Ground Pork vs. USDA/FSIS-MLG

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX System Method		USDA/ FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed			
18 Hr Primary Enrichment—8 Hr RV-R10 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38–1.16)	20	9	9	9	0.0	0.0
18 Hr Primary Enrichment—24 Hr RV-R10 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38–1.16)	20	9	9	9	0.0	0.0
24 Hr Primary Enrichment—8 Hr RV-R10 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38–1.16)	20	9	9	9	0.0	0.0
24 Hr Primary Enrichment—24 Hr RV-R10 Enrichment									
Control	N/A	2.4×10^7	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Montevideo ATCC 8387**		0.66 (0.38–1.16)	20	9	9	9	0.0	0.0

*Includes 95% confidence intervals

Table 13: 3M Petrifilm SALX System Summary Data Table for Cooked Chicken Nuggets vs. USDA/FSIS-MLG

Inoculation Level	Inoculating Organism	APC CFU/g	MPN/25g*	Total Samples	3M Petrifilm SALX System Method		USDA/ FSIS-MLG	X^2 Alternative Confirmation	X^2 Traditional Confirmation
					Presumptive	Confirmed			
18 Hr Primary Enrichment									
Control	N/A	2.4×10^2	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Typhimurium ATCC 14028**		0.03 (0.01–0.08)	20	6	6	6	0.0	0.0
24 Hr Primary Enrichment									
Control	N/A	2.4×10^2	< 0.075	5	0	0	0	–	–
0.2–2 CFU/ Test Portion	***S. enterica* ser. Typhimurium ATCC 14028**		0.03 (0.01–0.08)	20	6	6	6	0.0	0.0

*Includes 95% confidence intervals