



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**042001**

The AOAC Research Institute hereby certifies the test kit known as:

**Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) *Salmonella* Assay**

manufactured by

**Applied Food Diagnostics, Inc.**

**387 Hazle Street**

**Nuremberg, PA**

**USA**

This method has been evaluated as a single-site method in the AOAC<sup>®</sup> *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above-mentioned method for a period of one calendar year from the date of this certificate (January 01, 2021 – December 31, 2021). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

\_\_\_\_\_  
January 01, 2021

Date

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| <b>METHOD AUTHORS</b><br>Thomas Lonczynski and Laura Cowin  | <b>SUBMITTING COMPANY</b><br>Applied Food Diagnostics, Inc.<br>387 Hazle Street<br>Nuremberg, PA USA   |
| <b>KIT NAME(S)</b><br>Simultaneous Multiplex Real Time PCR (SIMUL-qPCR) <i>Salmonella</i> Assay<br>(Single source service-based method)   | <b>CATALOG NUMBER</b><br>SMRT-SAL-096  |
| <b>INDEPENDENT LABORATORY</b><br>WBA Analytical Laboratories<br>3609 Johnson Road<br>Springdale, AR   | <b>AOAC EXPERTS AND PEER REVIEWERS</b><br>Thomas Hammack <sup>1</sup> , Michael Brodsky <sup>2</sup> , Wayne Ziemer <sup>3</sup><br><sup>1</sup> US FDA CFSAN, College Park, MD, USA<br><sup>2</sup> Brodsky Consultants, Ontario, CANADA<br><sup>3</sup> Loganville, GA, USA  |
| <b>APPLICABILITY OF METHOD</b><br>Analyte – <i>Salmonella</i> species<br><br>Matrixes – (MLG 4.09) Raw beef trim (375 g, ~80% lean), raw ground beef (375 g, ~80% lean), raw ground poultry (375 g), ready-to-eat cooked poultry (375 g), pasteurized liquid eggs (100 g), frankfurters/sausages (25 g), poultry carcass rinses (FDA BAM Ch 5) - dry pet food (375 g), peanut butter (25 g), stainless steel (4" x 4", 1" x 1"), plastic (1" x 1"), rubber (1" x 1"), ceramic tile (1" x 1"), and sealed concrete (1" x 1")<br><br>Performance claims - Performance equivalent to that of the U. S. Department of Agriculture-Food Safety and Inspection Service <i>Microbiology Laboratory Guidebook</i> (MLG), 4.09, Isolation and Identification of <i>Salmonella</i> from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges (2) for raw beef trim, raw ground beef, raw ground poultry, RTE cooked poultry, frankfurters, pasteurized liquid eggs and poultry carcass rinses, and the U.S. Food and Drug Administration <i>Bacteriological Analytical Manual</i> (BAM) Chapter 5 (3) for dry pet food, peanut butter and environmental surface sponges/swabs. Lysates from enriched samples can be tested individually or in a pooled format. In the pooled format, up to 10 lysates can be combined. The pooled format is not indicated for pasteurized liquid egg. | <b>REFERENCE METHODS</b><br>U. S Department of Agriculture Food Safety and Inspection Service <i>Microbiology Laboratory Manual</i> , Isolation and Identification of <i>Salmonella</i> from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges, 4.09 (2)<br>Food and Drug Administration <i>Bacteriological Analytical Manual</i> , <i>Salmonella</i> , Chapter 5 (3) |
| <b>ORIGINAL CERTIFICATION DATE</b><br>April 07, 2020  | <b>CERTIFICATION RENEWAL RECORD</b><br>Renewed annually through December 2021  |
| <b>METHOD MODIFICATION RECORD</b><br>NONE   | <b>SUMMARY OF MODIFICATION</b><br>NONE   |
| Under this AOAC® <i>Performance Tested</i> <sup>SM</sup> License Number, 042001 this method is distributed by:<br>NONE  | Under this AOAC® <i>Performance Tested</i> <sup>SM</sup> License Number, 042001 this method is distributed as:<br>NONE   |

## PRINCIPLE OF THE METHOD (1)

This protocol is a multifaceted approach to the detection of *Salmonella* species in selected food products and environmental samples. Specifically formulated media are utilized for enriching samples followed by rapid (qPCR) detection procedures. Buffered Peptone Water (BPW) contains necessary nutritional components for the growth of *Salmonella*. Enterohemorrhagic *E. coli* Recovery and Enrichment Broth (EREB) combine those nutritional components with additional ingredients that are necessary to selectively improve recovery and growth of *Salmonella*. The selective agents present in EREB have been optimized to efficiently inhibit competing bacterial flora without affecting the growth of *Salmonella* species. BPW and EREB are formulated for buffering capacity to ensure growth in a variety of matrixes.

The sample is enriched at a specific temperature. Detection procedures occur after a specified minimal enrichment time. qPCR detection occurs prior to the pooled qPCR protocol and cultural protocol due to the increased sensitivity of this technology. Enrichment continues to allow additional growth ensuring the sensitivity of the pooled sample protocol. The extended enrichment time also ensures culturally viable recoverable *Salmonella* cells on the detection plate. For qPCR amplification and detection, forward and reverse primers hybridize to a unique sequence *Salmonella* genomic DNA. A fluorogenic probe consisting of a DNA probe labeled with a 5'-dye and a 3'-quencher is included in the same reaction mixture. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on the real-time PCR instrument. Two unique primer and probe mixtures specific for *Salmonella* are present in this assay.

## DISCUSSION OF THE VALIDATION STUDY (1)

### *Inclusivity and Exclusivity*

Of the 100 inclusivity strains analyzed by the SIMUL-qPCR *Salmonella* Assay, all 100 inclusivity strains were correctly detected by the SIMUL-qPCR *Salmonella* assay kit. Of the 30 exclusivity strains, none were detected by the SIMUL-qPCR method. Initially two of the exclusivity isolates gave a positive PCR call (*Shigella flexneri* and *Providencia stuartii*). After re-growing the isolates per the recommended SIMUL-qPCR method enrichment procedure and re-analyzing, all results were negative.

### *Real-Time Stability Study*

The results from the real-time product consistency and stability study showed that there were no statistically significant differences by POD analysis between the recently manufactured lots and those halfway through the expiration period or the recently manufactured lots and those nearing expiration. The study verified the one-year shelf life of the SIMUL-qPCR *Salmonella* Assay.

### *Robustness Study*

The results from the robustness study showed that there was no statistically significant difference by POD analysis when small alterations are made to the protocol. Increasing or decreasing enrichment time, volume of the extraction reagent, and volume of extracted DNA sample slightly did not affect the performance of the *Salmonella* Assay.

### *Matrix Study*

Results from both the method developer and independent studies of the SIMUL-qPCR *Salmonella* Assay for the food and environmental samples are outlined in Tables 4–11. Throughout the study, the method developer had difficulty achieving fractional results, and repeat attempts had to be made to spike at the correct inoculation levels. The method developer also saw issues with pooling at fractional levels. During the pooling process, it was determined that the pooled test portions had to incubate for an additional two hours for raw ground beef and raw beef trim in order to achieve the fractional results comparable to the reference method. Also, pooling was not possible for pasteurized liquid eggs. Environmental samples proved challenging to recover cells off the surface at low levels due to cell die-off. Multiple trials were run for some of the surfaces to achieve fractional levels.

During confirmation testing for the raw products, the method developer laboratory had issues with competing flora growing on plates. Colonies had to be re-streak for further isolation to obtain the intended target.

For raw beef trim, differences were seen in the number of presumptive positive results between the 10 h time point and the 18 h time point. Differences were also seen in the number of presumptive positive results between the individual and pooled samples. This was caused by late C<sub>q</sub> calls that dropped off once the positive samples were pooled with non-inoculated samples. The 10 h time point for individual samples had five less presumptive than confirmed results. However, this was not found to be statistically significant. The 12 h pooled time point showed two less presumptive results compared to the confirmed results. At 18 h, the number of individual sample positives matched the number of confirmed positives. For the 18 h pooled samples, there were four less presumptive calls compared to the confirmed results. It's possible that the *Salmonella* were near the detection limit and could have been just under once the samples were pooled. In all instances, there was no statistically significant differences when compared by POD analysis. When comparing the SIMUL-qPCR *Salmonella* Assay confirmed positives to the FSIS MLG 4.09 reference method positives, there was no statistically significant differences between the candidate and reference method for raw beef trim. However, it is recommended that the raw ground beef and raw beef trim incubate longer than 10 hours. It is also recommended raw beef trim samples whose lysates will be pooled incubate for the full 18 hours.

For raw ground beef, at both the 10 h individual time point and the 12 h pooled time point, 8 samples were presumptive positive while 11 samples confirmed positive. At these time points the *Salmonella* could have been just under the detection limit. At the 18 h time point for both individual and pooled samples, the number of presumptive positives and confirmed positives for the candidate method matched. The FSIS MLG 4.09 reference method had 14 confirmed positives compared to the 11 confirmed positives for the candidate method. POD analysis showed no statistically significant data between the two methods.

For raw ground poultry, both the candidate and reference method only had confirmed positives for four of the five high samples. The candidate method had one low inoculation level sample that did not call on the PCR but did confirm as positive for *Salmonella*. However, when the samples were pooled together, the same number of presumptive and confirmed positives occurred for the candidate method. For both individual and pooled samples, there were no statistically significant differences between the candidate presumptive versus confirmed results or between the candidate and FSIS MLG 4.09 reference method confirmed results when compared by POD analysis.

For ready to eat cooked poultry, both the individual and pooled samples had one less presumptive result compared to the confirmed result. However, POD analysis showed that the differences in the data was not statistically significant. The FSIS MLG 4.09 reference method had two more confirmed positives than the candidate method which had no statistical significance.

For frankfurters, the candidate method had 3 presumptive positive results for the individual samples that did not confirm when biochemical confirmation was performed. The 3 presumptive results had late C<sub>q</sub> calls, which was found to be difficult to culturally confirm due to low levels of *Salmonella* target cells. The pooled samples had the same number of presumptive results compared to confirmed results. In both cases, the results showed no statistically significant difference between presumptive and confirmed results. The reference method had fewer positive results than the candidate method for both individual and pooled samples, but difference was not statistically significant.

For dry pet food, there were difficulties with recovering the cells. It was discovered that pre-warming the BPW prior to adding the test portion caused increased die off. When the enrichment media was not pre-warmed, the cells were recoverable. For individual samples, the same number of presumptive and confirmed results occurred. For pooled samples, two of the confirmed samples did not yield a positive result on the candidate method. This was due to later C<sub>q</sub> calls for the individual samples that dropped off when combined with negative samples. However, this did not lead to any statistical significance. The FDA BAM reference method had two more confirmed positives than the candidate method, but this did not lead to any statistically significant differences.

For peanut butter, for both the individual and pooled samples, all the presumptive positive results for the candidate method were confirmed. The FDA BAM Chapter 5 reference method did have more confirmed positives than the candidate method, but it did not lead to any statically significant differences.

For pasteurized liquid eggs, the citric acid in the eggs caused PCR inhibition. The samples had to be diluted for IAC inhibition to not occur. Because of this, pooling could not be done on these samples. Out of the twenty fractional level samples, nine were presumptive on the candidate method. However, twelve of those samples confirmed. When the data was compared using the POD analysis, it was found that this difference was not statistically significant. Twelve samples also confirmed for the reference method, showing no statistical significance in the data.

For the poultry carcass rinses, the presumptive and confirmed results for both individual and pooled samples matched. The FSIS MG 4.09 reference method had three more confirmed positives than the candidate method. However, after POD analysis, there was no statistically significant differences between the two methods.

For stainless steel, a 1" x 1" surface area was tested using a swab and a 4" x 4" surface area was tested using a sponge via the candidate method. For the high inoculation level, the swab detected all five high positive samples, which all confirmed. The sponge detected four of the five high, in which all five confirmed. For the low inoculation level, the swab detected 7 presumptive positive results in the 1" x 1" area, and all 7 confirmed. The sponge detected 9 presumptive positive results, but only 8 confirmed. The one presumptive that did not confirm had a late C<sub>q</sub> call which was not culturally viable. The FDA BAM Chapter 5 method had 10 confirmed results, which showed no statistically significant difference when analyzed using POD analysis.

The plastic was swabbed in a 1" x 1" surface area. Four samples had presumptive positive calls, and all four confirmed. The FDA BAM Chapter 5 method had five samples confirm positives, leading to no statistically significant differences between the two methods.

The rubber was also swabbed in a 1" x 1" surface area. For the high inoculation level, four of the five samples were presumptive and four confirmed. For the low-level inoculation, four samples were presumptive positive on the PCR, and five samples confirmed. For the FDA BAM Chapter 5 method, 9 samples confirmed. When performing the POD analysis on the two methods, no statistically significant differences were observed.

For ceramic, a 1" x 1" surface area was tested. The candidate method had all five high inoculation samples call presumptive and confirm compared to four for the reference method. For the low inoculation level, the candidate had fourteen presumptive calls, and all fourteen confirmed. The FDA BAM Chapter 5 method had eleven confirmed positives. For ceramic, the candidate method had more confirmed positives than the reference method, and POD analysis showed no statistical difference.

For concrete, 1" x 1" surface area was also swabbed. For the high inoculation set, the candidate method had four presumptive positive results that confirmed, and the FDA BAM Chapter 5 reference method also four confirmed positives. For the low inoculation set, the candidate method had four presumptive positive results that all confirmed, and the reference method had five confirmed results. POD analysis did not show any statistically significant differences in the two methods.

#### *Independent Laboratory Studies*

For the SIMUL-qPCR method of raw ground beef, at the 10 h individual enrichment point, as well as 12 h and 18 h pooled enrichment points, 4 out of 20 samples were positive. At the 18 h individual enrichment time point, 5 out of 20 samples were positive. Four out of 20 test portions confirmed positive. For the reference method, 5 out of 20 test portions confirmed positive. All five of the high inoculation set were positive and all five of the uninoculated sets were negative for both the candidate and reference methods.

For dry pet food, at both the 16 h individual and pooled enrichment time point, 9 out of 20 test portions were presumptive positive. All nine samples culturally confirmed as well. For the reference method, 10 out of 20 test portions confirmed positive. All five of the high inoculation set were positive and all five of the uninoculated sets were negative for both the candidate and reference methods.

For the stainless-steel swabs, at the 16 h individual time point 14 out of 20 test portions were positive. At the 16 h pooled time point, 13 out of 20 were positive. Fourteen were confirmed positive. The one pooled portion that was not PCR positive was most likely close to the detection limit. For the reference method, 12 out of 20 samples confirmed positive. For both the candidate and reference methods, all five of the high inoculation set were positive and all five of the uninoculated sets were negative.

There were no statistically significant differences found between the candidate presumptive versus confirmed results at any time point, and no significant differences found between the candidate and reference methods for any of the matrixes.

**Table 1. Inclusivity List – *Salmonella* Strains (1)**

| No. | Genus             | Species                                  | Serovar                            | Source                 | Origin             | SIMUL-qPCR Result - BPW | SIMUL-qPCR Result - EREB |
|-----|-------------------|--|------------------------------------|------------------------|--------------------|-------------------------|--------------------------|
| 1   | <i>Salmonella</i> | <i>enterica</i> subsp. <i>houtenae</i>   | 1, 40:g, z51:-                     | SGSC <sup>o</sup> 3120 | Unknown, Tonga     | +                       | +                        |
| 2   | <i>Salmonella</i> | <i>enterica</i> subsp. <i>indica</i>     | 11:b:e, n, x                       | SGSC 3118              | Unknown            | +                       | +                        |
| 3   | <i>Salmonella</i> | <i>enterica</i> subsp. <i>houtenae</i>   | 16:z4,z32:-                        | SGSC 3086              | Human, Illinois    | +                       | +                        |
| 4   | <i>Salmonella</i> | <i>enterica</i> subsp. <i>diarizonae</i> | 38[k]:z35:-                        | SGSC 3069              | Human, California  | +                       | +                        |
| 5   | <i>Salmonella</i> | <i>enterica</i> subsp. VII               | 40:z4, z24:-                       | SGSC 3121              | Human, Florida     | +                       | +                        |
| 6   | <i>Salmonella</i> | <i>enterica</i> subsp. <i>salamae</i>    | 42:f:g,t:-                         | SGSC 3047              | Unknown            | +                       | +                        |
| 7   | <i>Salmonella</i> | <i>enterica</i> subsp. <i>indica</i>     | 45:a:e, n, x                       | SGSC 3116              | Unknown, India     | +                       | +                        |
| 8   | <i>Salmonella</i> | <i>enterica</i> subsp. IV                | 45a,b:g,z32:-                      | SGSC 3074              | Animal, Canal Zone | +                       | +                        |
| 9   | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Agona (B)                          | SGSC 2458              | Unknown, Peru      | +                       | +                        |
| 10  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Anatum (E <sub>1</sub> )           | SGSC 2459              | Human, Washington  | +                       | +                        |
| 11  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>arizonae</i>   | 62:z4,z23: - -                     | SGSC 3061              | Corn snake, Oregon | +                       | +                        |
| 12  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>arizonae</i>   | 62:z36: - -                        | SGSC 3063              | Human, California  | +                       | +                        |
| 13  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>arizonae</i>   | 51:z(4),z(23):(-)                  | SGSC 2426              | Unknown            | +                       | +                        |
| 14  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>salamae</i>    | II 17:g,t:[e,n,x,z <sub>15</sub> ] | SGSC 2415              | Unknown            | +                       | +                        |
| 15  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>salamae</i>    | II 6,7:b:e,n,x:z <sub>42</sub>     | SGSC 2414              | Unknown            | +                       | +                        |
| 16  | <i>Salmonella</i> | <i>bongori</i>                           | 48:z35:-                           | SGSC 2556              | Unknown            | +                       | +                        |
| 17  | <i>Salmonella</i> | <i>bongori</i>                           | 66:z41: - -                        | SGSC 3100              | Frog               | +                       | +                        |
| 18  | <i>Salmonella</i> | <i>bongori</i>                           | 48:z41: - -                        | SGSC 3103              | Parakeet, USA      | +                       | +                        |
| 19  | <i>Salmonella</i> | <i>bongori</i>                           | 66:z(41):(-)                       | SGSC 2429              | Unknown            | +                       | +                        |
| 20  | <i>Salmonella</i> | <i>bongori</i>                           | 66:z41:-                           | SGSC 2557              | Unknown            | +                       | +                        |
| 21  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Choleraesuis                       | SGSC 2461              | Swine, Minnesota   | +                       | +                        |
| 22  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Decatur                            | SGSC 2465              | Unknown, France    | +                       | +                        |
| 23  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Derby (B)                          | SGSC 2466              | Avian, Oklahoma    | +                       | +                        |
| 24  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>diarizonae</i> | 6,7:1,v:z(53)                      | SGSC 2427              | Unknown            | +                       | +                        |
| 25  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Dublin (D <sub>1</sub> )           | SGSC 3611              | Unknown            | +                       | +                        |

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|    |                   |  |                               |                               |                                       |   |   |
|----|-------------------|--|-------------------------------|-------------------------------|---------------------------------------|---|---|
| 26 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Bredeney (B)                  | SGSC 4931                     | Unknown                               | + | + |
| 27 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Duisberg                      | SGSC 2472                     | Unknown, Scotland                     | + | + |
| 28 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Emek (C <sub>3</sub> )        | SGSC 2477                     | Unknown, Israel                       | + | + |
| 29 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Newport (C <sub>2</sub> )     | ATCC <sup>b</sup> 6962        | Food poisoning fatality               | + | + |
| 30 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Enteritidis (D <sub>1</sub> ) | ATCC 13076                    | Unknown                               | + | + |
| 31 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Montevideo (C <sub>1</sub> )  | ATCC 8387                     | Unknown                               | + | + |
| 32 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Schwarzengrund (B)            | BEI <sup>c</sup> NR-28796     | Human, Oregon                         | + | + |
| 33 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Typhi (D <sub>1</sub> )       | BEI NR-513                    | USA                                   | + | + |
| 34 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Typhimurium (B)               | USDA ERRC <sup>d</sup> B-4211 | Ames, B. N. (U California, Davis, CA) | + | + |
| 35 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Poona (G <sub>1</sub> )       | SGSC 4934                     | Unknown                               | + | + |
| 36 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Blockley C <sub>2</sub> )     | SGSC 4935                     | Unknown                               | + | + |
| 37 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Livingstone (C <sub>1</sub> ) | SGSC 4937                     | Unknown                               | + | + |
| 38 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Hvitittingfoss (I)            | SGSC 4947                     | Unknown                               | + | + |
| 39 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Bareilly (C <sub>1</sub> )    | SGSC 4949                     | Unknown                               | + | + |
| 40 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Mbandaka (C <sub>1</sub> )    | SGSC 2958                     | Unknown                               | + | + |
| 41 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Setubal (60)                  | SGSC 2567                     | Unknown, Mississippi                  | + | + |
| 42 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>indica</i>   | Ferlac (VI 1,6,14,25)         | SGSC 2581                     | Unknown                               | + | + |
| 43 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Flint (IV 50)                 | SGSC 2554                     | Unknown                               | + | + |
| 44 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Gallinarum (D <sub>1</sub> )  | SGSC 2478                     | Human, Connecticut                    | + | + |
| 45 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Haifa (B)                     | SGSC 2479                     | Unknown, Scotland                     | + | + |
| 46 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Heidelberg (B)                | SGSC 2480                     | Chicken, Pennsylvania                 | + | + |
| 47 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>houtenae</i> | 45:g,z(51):(-)                | SGSC 2428                     | Unknown                               | + | + |
| 48 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Indiana (B)                   | SGSC 2482                     | Unknown, Scotland                     | + | + |
| 49 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>indica</i>   | 1,6,14,25:a:e,n,x             | SGSC 2430                     | Unknown                               | + | + |
| 50 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Infantis (C <sub>1</sub> )    | SGSC 2483                     | Human, North Carolina                 | + | + |
| 51 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Argentina (IV 6,7)            | SGSC 2555                     | Unknown                               | + | + |
| 52 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Paratyphi B var. Java         | SGSC 4951                     | Unknown                               | + | + |
| 53 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i> | Krefeld (E <sub>4</sub> )     | SGSC 4945                     | Unknown, France                       | + | + |

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|    |                   |  |                               |              |                            |   |   |
|----|-------------------|--|-------------------------------|--------------|----------------------------|---|---|
| 54 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Malawi (66)                   | SGSC 2577    | Unknown                    | + | + |
| 55 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Maregrosso (V 66)             | SGSC 2578    | Unknown                    | + | + |
| 56 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Miami (D <sub>1</sub> )       | SGSC 2486    | Human, French Guiana       | + | + |
| 57 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>salamae</i>    | 58:d:z                        | SGSC 3039    | Human, Massachusetts       | + | + |
| 58 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Muenchen (C <sub>2</sub> )    | SGSC 2490    | Human, France              | + | + |
| 59 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Naestved (D <sub>1</sub> )    | SGSC 3612    | Unknown                    | + | + |
| 60 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Newport (C <sub>2</sub> )     | SGSC 2493    | Human, North Carolina      | + | + |
| 61 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>diarizonae</i> | 50:k:z                        | SGSC 3068    | Human, Oregon              | + | + |
| 62 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Ohio (C <sub>1</sub> )        | SGSC 4943    | Unknown                    | + | + |
| 63 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Panama (D <sub>1</sub> )      | SGSC 3583    | Unknown                    | + | + |
| 64 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Paratyphi A                   | SGSC 4568    | Unknown                    | + | + |
| 65 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Paratyphi B                   | SGSC 4567    | Unknown                    | + | + |
| 66 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Paratyphi C                   | SGSC 2290    | Unknown                    | + | + |
| 67 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Oranienburg (C <sub>1</sub> ) | BEI NR-171   | Human, Illinois            | + | + |
| 68 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Javiana (D <sub>1</sub> )     | BEI NR-4296  | Human, Pennsylvania        | + | + |
| 69 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Pullorum (D <sub>1</sub> )    | SGSC 2508    | Unknown, Germany           | + | + |
| 70 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Reading (B)                   | SGSC 2510    | Unknown, Scotland          | + | + |
| 71 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Rissen (C <sub>1</sub> )      | SGSC 4941    | Unknown                    | + | + |
| 72 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Rubislaw (11)                 | SGSC 2511    | Unknown, Laboratory Strain | + | + |
| 73 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | S.4, 5, 12:i:-                | SGSC 4956    | Unknown                    | + | + |
| 74 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Saintpaul (B)                 | SGSC 2512    | Human, Texas               | + | + |
| 75 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>salamae</i>    | 1,9,12:1,w:e,n,x              | SGSC 2425    | Unknown                    | + | + |
| 76 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Abortusovis (B)               | BEI NR-13556 | Unknown, France            | + | + |
| 77 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Sendai (D <sub>1</sub> )      | SGSC 2515    | Human, California          | + | + |
| 78 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Senftenberg (E <sub>4</sub> ) | SGSC 2516    | Chicken, Maryland          | + | + |
| 79 | <i>Salmonella</i> | spp.                                     | 44:z39: - -                   | SGSC 3105    | Food, Ghana                | + | + |
| 80 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Stanley (B)                   | SGSC 2517    | Unknown, Scotland          | + | + |
| 81 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Stanleyville (B)              | SGSC 2518    | Unknown, Scotland          | + | + |

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|     |                   |  |                               |               |                      |   |   |
|-----|-------------------|--|-------------------------------|---------------|----------------------|---|---|
| 82  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Tennessee (C <sub>1</sub> )   | SGSC 4939     | Unknown              | + | + |
| 83  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Thompson (C <sub>1</sub> )    | SGSC 2519     | Human, Florida       | + | + |
| 84  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Hadar (C <sub>2</sub> )       | BEI NR-28799  | Human, Massachusetts | + | + |
| 85  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Virchow (C <sub>1</sub> )     | BEI NR-28801  | Human, Connecticut   | + | + |
| 86  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Kentucky (C <sub>3</sub> )    | BEI NR-28795  | Human, Wisconsin     | + | + |
| 87  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Typhisuis (B)                 | SGSC 2526     | Swine, California    | + | + |
| 88  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Vrindaban (VI 45)             | SGSC 2582     | Unknown              | + | + |
| 89  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Wassenaar (IV 50)             | SGSC 2576     | Unknown              | + | + |
| 90  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Weltevreden (E <sub>1</sub> ) | SGSC 4929     | Unknown              | + | + |
| 91  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Wien (B)                      | SGSC 2529     | Human, France        | + | + |
| 92  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>enterica</i>   | Brandenberg (B)               | SGSC 2460     | Unknown, Scotland    | + | + |
| 93  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>indica</i>     | 45:a:e,n,x                    | ATCC BAA-1578 | India                | + | + |
| 94  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>diarizonae</i> | 6,7:l,v:z53                   | ATCC 43973    | Unknown              | + | + |
| 95  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>dublin</i>     | 1,9,12,[Vi]:g,p:--            | SGSC 2471     | Unknown, Thailand    | + | + |
| 96  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>houtenae</i>   | 11:z4,z23:                    | ATCC 15788    | Water, Bonaire       | + | + |
| 97  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>salamae</i>    | 9,46:z4z24:z39:z42            | ATCC 15793    | Lizard, Netherlands  | + | + |
| 98  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>salamae</i>    | 55:k:z39                      | ATCC 700148   | Unknown              | + | + |
| 99  | <i>Salmonella</i> | <i>enterica</i> subsp. <i>wien</i>       | 1,4,12,27:b:l,w               | SGSC 2528     | Human, France        | + | + |
| 100 | <i>Salmonella</i> | <i>enterica</i> subsp. <i>diarizonae</i> | IIIb 47:i:z53:z57             | ATCC 12325    | CDC                  | + | + |

<sup>a</sup>Genetic Stock Centre, University of Calgary, Canada.

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

<sup>c</sup>BEI Resources, Manassas, VA.

<sup>d</sup>United States Department of Agriculture Eastern Regional Research Center, Wyndmoor, PA



**Table 2. Exclusivity List – Non-Salmonella Strains (1)**

| No. | Genus                 | Species                                    | Source                         | Origin                    | SIMUL-qPCR Result |
|-----|-----------------------|--|--------------------------------|---------------------------|-------------------|
| 1   | <i>Bacillus</i>       | <i>cereus</i>                              | BEI <sup>a</sup> NR-2488       | Blood                     | -                 |
| 2   | <i>Bacillus</i>       | <i>subtilis</i>                            | BEI NR-607                     | Unknown                   | -                 |
| 3   | <i>Bacillus</i>       | <i>pumilus</i>                             | BEI NR-605                     | Unknown                   | -                 |
| 4   | <i>Carnobacterium</i> | <i>divergens</i>                           | ATCC <sup>b</sup> 35677        | Vacuum-packed minced beef | -                 |
| 5   | <i>Citrobacter</i>    | <i>freundii</i>                            | ATCC 43864                     | Unknown                   | -                 |
| 6   | <i>Citrobacter</i>    | <i>koseri</i>                              | SGSC <sup>c</sup> 5610         | Clinical                  | -                 |
| 7   | <i>Ewingella</i>      | <i>americana</i>                           | SGSC 5640                      | Human feces               | -                 |
| 8   | <i>Enterobacter</i>   | <i>cloacae</i>                             | SGSC 5330                      | Unknown                   | -                 |
| 9   | <i>Enterobacter</i>   | <i>aerogenes</i>                           | SGSC 5347                      | Unknown                   | -                 |
| 10  | <i>Enterobacter</i>   | <i>taylorae</i>                            | SGSC 5283                      | Unknown                   | -                 |
| 11  | <i>Escherichia</i>    | <i>coli</i> O157:H7                        | SGSC 5982                      | Unknown                   | -                 |
| 12  | <i>Escherichia</i>    | <i>coli</i> O1:K1:H(-)                     | SGSC 2811                      | Unknown                   | -                 |
| 13  | <i>Escherichia</i>    | <i>fergusoni</i>                           | SGSC 5718                      | Human feces               | -                 |
| 14  | <i>Klebsiella</i>     | <i>oxytoca</i>                             | SGSC 5366                      | Unknown                   | -                 |
| 15  | <i>Klebsiella</i>     | <i>pneumoniae</i> subsp. <i>pneumoniae</i> | SGSC 5926                      | Unknown                   | -                 |
| 16  | <i>Klebsiella</i>     | <i>planticola</i>                          | SGSC 5929                      | Radish root               | -                 |
| 17  | <i>Pseudomonas</i>    | <i>aeruginosa</i>                          | BEI NR-48982                   | Human                     | -                 |
| 18  | <i>Proteus</i>        | <i>mirabilis</i>                           | SGSC 5445                      | Unknown                   | -                 |
| 19  | <i>Providencia</i>    | <i>stuartii</i>                            | SGSC 5639                      | Clinical                  | + <sup>d</sup>    |
| 20  | <i>Serratia</i>       | <i>marcescens</i>                          | SGSC 5354                      | Unknown                   | -                 |
| 21  | <i>Serratia</i>       | <i>odorifera</i>                           | SGSC 5720                      | Sputum                    | -                 |
| 22  | <i>Shigella</i>       | <i>flexneri</i>                            | SGSC 5577                      | Unknown                   | + <sup>d</sup>    |
| 23  | <i>Streptococcus</i>  | <i>pyogenes</i>                            | BEI NR-51272                   | Human                     | -                 |
| 24  | <i>Staphylococcus</i> | <i>aureus</i>                              | BEI NR-46412                   | Urine                     | -                 |
| 25  | <i>Streptococcus</i>  | <i>bovis</i>                               | USDA <sup>e</sup> ERRC B-23788 | Diseased bovine tissue    | -                 |
| 26  | <i>Yersinia</i>       | <i>enterocolitica</i>                      | USDA ERRC B-41479              | Ground beef               | -                 |
| 27  | <i>Listeria</i>       | <i>monocytogenes</i> 4c                    | BEI NR-111                     | Chicken                   | -                 |
| 28  | <i>Listeria</i>       | <i>marthii</i>                             | BEI NR-9582                    | River water               | -                 |
| 29  | <i>Escherichia</i>    | <i>coli</i> O121:H19                       | BEI NR-17630                   | Human                     | -                 |
| 30  | <i>Escherichia</i>    | <i>hermanii</i>                            | SGSC 5715                      | Human toe                 | -                 |

<sup>a</sup>BEI Resources, Manassas, VA.

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

<sup>c</sup>*Salmonella* Genetic Stock Centre, University of Calgary, Canada.

<sup>d</sup>Strain was positive by SIMUL-qPCR *Salmonella* assay when grown overnight in non-selective enrichment broth but negative under the recommended enrichment conditions (BPW and EREB).

<sup>e</sup>United States Department of Agriculture Eastern Regional Research Center, Windsor, PA.

**Table 4. SIMUL-qPCR *Salmonella* Assay Presumptive vs. Confirmed Results for Raw Ground Beef and Raw Beef Trim – Individual samples (1)**

| Matrix                                    | Strain   | MPN <sup>g</sup> /test portion | SIMUL-qPCR <i>Salmonella</i> Presumptive |                |                                |            | SIMUL-qPCR Top <i>Salmonella</i> Confirmed |                                |            |                                 |                     |
|---|--|--------------------------------|--|----------------|--------------------------------|------------|--|--------------------------------|------------|---------------------------------|---------------------|
|   |  |                                | N <sup>b</sup>                           | x <sup>c</sup> | POD <sub>CP</sub> <sup>d</sup> | 95% CI     | x  | POD <sub>CC</sub> <sup>e</sup> | 95% CI     | dPOD <sub>CP</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
| Ground beef<br>375 g<br>10 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A <sup>h</sup>               | 5  | 0              | 0.00                           | 0.00, 0.43 | 0  | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.63 (1.09, 2.90)              | 20                                       | 8              | 0.40                           | 0.22, 0.61 | 11   | 0.55                           | 0.34, 0.74 | -0.15                           | -0.41, 0.15         |
|   |  | 9.26 (3.80, 22.55)             | 5  | 4              | 0.80                           | 0.38, 1.00 | 5  | 1.00                           | 0.57, 1.00 | -0.20                           | -0.62, 0.28         |
| Ground beef <sup>i</sup><br>375 g<br>10 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A                            | 5  | 0              | 0.00                           | 0.00, 0.43 | 0  | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 0.45 (0.23, 0.76)              | 20                                       | 5              | 0.25                           | 0.11, 0.47 | 4  | 0.20                           | 0.08, 0.42 | 0.00                            | -0.13, 0.13         |
|   |  | 4.46 (2.13, 9.31)              | 5  | 4              | 0.80                           | 0.38, 1.00 | 5  | 1.00                           | 0.57, 1.00 | -0.20                           | -0.62, 0.28         |
| Beef trim<br>375 g<br>10 h                | <i>S. Infantis</i> , C <sub>1</sub><br>(SGSC 2483) | N/A                            | 5  | 0              | 0.00                           | 0.00, 0.43 | 0  | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 0.723 (0.43, 1.17)             | 20                                       | 8              | 0.40                           | 0.22, 0.61 | 13   | 0.65                           | 0.43, 0.82 | -0.25                           | -0.50, 0.05         |
|   |  | 7.275 (3.36, 15.74)            | 5  | 5              | 1.00                           | 0.57, 1.00 | 5  | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Ground beef<br>375 g<br>18 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A <sup>h</sup>               | 5  | 0              | 0.00                           | 0.00, 0.43 | 0  | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.63 (1.09, 2.90)              | 20                                       | 11             | 0.55                           | 0.34, 0.74 | 11   | 0.55                           | 0.34, 0.74 | 0.00                            | -0.28, 0.28         |
|   |  | 9.26 (3.80, 22.55)             | 5  | 5              | 1.00                           | 0.57, 1.00 | 5  | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Ground beef <sup>i</sup><br>375 g<br>18 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A                            | 5  | 0              | 0.00                           | 0.00, 0.43 | 0  | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 0.45 (0.23, 0.76)              | 20                                       | 5              | 0.25                           | 0.11, 0.47 | 4  | 0.20                           | 0.08, 0.42 | 0.05                            | -0.11, 0.21         |
|   |  | 4.46 (2.13, 9.31)              | 5  | 5              | 1.00                           | 0.57, 1.00 | 5  | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Beef trim<br>375 g<br>18 h                | <i>S. Infantis</i> , C <sub>1</sub><br>(SGSC 2483) | N/A                            | 5  | 0              | 0.00                           | 0.00, 0.43 | 0  | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 0.723 (0.43, 1.17)             | 20                                       | 13             | 0.65                           | 0.43, 0.82 | 13   | 0.65                           | 0.43, 0.82 | 0.00                            | -0.28, 0.28         |
|   |  | 7.275 (3.36, 15.74)            | 5  | 5              | 1.00                           | 0.57, 1.00 | 5  | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |

<sup>g</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

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

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result 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>Not applicable.

<sup>i</sup>Matrix tested by the independent laboratory.

**Table 5. SIMUL-qPCR *Salmonella* Assay vs. Reference Method Results for Raw Ground Beef and Raw Beef Trim – Individual samples (1)**

| Matrix                                    | Strain   | MPN <sup>a</sup> /test portion | N <sup>b</sup> | SIMUL-qPCR <i>Salmonella</i> Results Confirmed |                               |            | Reference Method Results (MLG 4.09) |                               |            |                                |                     |
|---|--|--------------------------------|----------------|--|-------------------------------|------------|-------------------------------------|-------------------------------|------------|--------------------------------|---------------------|
|   |  |                                |                | x <sup>c</sup>                                 | POD <sub>c</sub> <sup>d</sup> | 95% CI     | x                                   | POD <sub>R</sub> <sup>e</sup> | 95% CI     | dPOD <sub>c</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
| Ground beef<br>375 g<br>10 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A <sup>h</sup>               | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 1.63 (1.09, 2.90)              | 20             | 8  | 0.40                          | 0.22, 0.61 | 14                                  | 0.70                          | 0.48, 0.85 | -0.30                          | -0.54, 0.01         |
|   |  | 9.26 (3.80, 22.6)              | 5              | 4  | 0.80                          | 0.38, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | -0.20                          | -0.62, 0.28         |
| Ground beef <sup>i</sup><br>375 g<br>10 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 0.45 (0.23, 0.76)              | 20             | 4  | 0.20                          | 0.08, 0.42 | 5                                   | 0.25                          | 0.11, 0.47 | -0.05                          | -0.30, 0.21         |
|   |  | 4.46 (2.13, 9.31)              | 5              | 4  | 0.80                          | 0.38, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | -0.20                          | -0.62, 0.28         |
| Beef trim<br>375 g<br>10 h                | <i>S. Infantis</i> , C <sub>1</sub><br>(SGSC 2483) | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 0.723 (0.43, 1.17)             | 20             | 8  | 0.40                          | 0.22, 0.61 | 13                                  | 0.65                          | 0.43, 0.82 | -0.25                          | -0.50, 0.05         |
|   |  | 7.275 (3.36, 15.7)             | 5              | 5  | 1.00                          | 0.57, 1.00 | 3                                   | 0.60                          | 0.23, 0.88 | 0.40                           | -0.12, 0.77         |
| Ground beef<br>375 g<br>18 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 1.63 (1.09, 2.90)              | 20             | 11   | 0.55                          | 0.34, 0.74 | 14                                  | 0.70                          | 0.48, 0.85 | -0.15                          | -0.41, 0.14         |
|   |  | 9.26 (3.80, 22.6)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Ground beef <sup>i</sup><br>375 g<br>18 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 0.45 (0.23, 0.76)              | 20             | 4  | 0.20                          | 0.08, 0.42 | 5                                   | 0.25                          | 0.11, 0.47 | -0.05                          | -0.30, 0.21         |
|   |  | 4.46 (2.13, 9.31)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Beef trim<br>375 g<br>18 h                | <i>S. Infantis</i> , C <sub>1</sub><br>(SGSC 2483) | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 0.723 (0.43, 1.17)             | 20             | 13   | 0.65                          | 0.43, 0.82 | 13                                  | 0.65                          | 0.43, 0.82 | 0.00                           | -0.28, 0.28         |
|   |  | 7.275 (3.36, 15.7)             | 5              | 5  | 1.00                          | 0.57, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

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

<sup>f</sup>dPOD<sub>c</sub> = Difference between the candidate method result and reference method result 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>Not applicable.

<sup>i</sup>Matrix tested by the independent laboratory.

**Table 6. SIMUL-qPCR *Salmonella* Assay Presumptive vs. Confirmed Results for Raw Ground Beef and Raw Beef Trim – Pooled lysates (1)**

| Matrix                                    | Strain   | MPN <sup>a</sup> /test portion | N <sup>b</sup> | SIMUL-qPCR <i>Salmonella</i> Presumptive |                                |            | SIMUL-qPCR <i>Salmonella</i> Confirmed |                                |            | dPOD <sub>CP</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
|---|--|--------------------------------|----------------|--|--------------------------------|------------|--|--------------------------------|------------|---------------------------------|---------------------|
|   |  |                                |                | x <sup>c</sup>                           | POD <sub>CP</sub> <sup>d</sup> | 95% CI     | x                                      | POD <sub>CC</sub> <sup>e</sup> | 95% CI     |                                 |                     |
| Ground beef<br>375 g<br>12 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A <sup>h</sup>               | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00,0.43  | 0.00                            | -0.43, 0.43         |
|   |  | 1.63 (1.09, 2.90)              | 20             | 8  | 0.40                           | 0.22, 0.61 | 11                                     | 0.55                           | 0.34, 0.74 | -0.15                           | -0.41, 0.15         |
|   |  | 9.26 (3.80, 22.6)              | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Ground beef <sup>i</sup><br>375 g<br>12 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A                            | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00,0.43  | 0.00                            | -0.43, 0.43         |
|   |  | 0.45 (0.23, 0.76)              | 20             | 4  | 0.20                           | 0.08, 0.42 | 4                                      | 0.20                           | 0.08, 0.42 | 0.00                            | -0.13, 0.13         |
|   |  | 4.46 (2.13, 9.31)              | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Beef trim<br>375 g<br>12 h                | <i>S. Infantis</i> , C <sub>1</sub> (SGSC<br>2483) | N/A                            | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 0.72 (0.43, 1.17)              | 20             | 11                                       | 0.55                           | 0.34, 0.74 | 13                                     | 0.65                           | 0.43, 0.82 | -0.10                           | -0.37, 0.19         |
|   |  | 7.28 (3.36, 15.7)              | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Ground beef<br>375 g<br>18 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A <sup>h</sup>               | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.63 (1.09, 2.90)              | 20             | 11                                       | 0.55                           | 0.34, 0.74 | 11                                     | 0.55                           | 0.34, 0.74 | 0.00                            | -0.28, 0.28         |
|   |  | 9.26 (3.80, 22.6)              | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Ground beef <sup>i</sup><br>375 g<br>18 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211)    | N/A                            | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00,0.43  | 0.00                            | -0.43, 0.43         |
|   |  | 0.45 (0.23, 0.76)              | 20             | 4  | 0.20                           | 0.08, 0.42 | 4                                      | 0.20                           | 0.08, 0.42 | 0.00                            | -0.13, 0.13         |
|   |  | 4.46 (2.13, 9.31)              | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Beef trim<br>375 g<br>18 h                | <i>S. Infantis</i> , C <sub>1</sub> (SGSC<br>2483) | N/A                            | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 0.723 (0.43, 1.17)             | 20             | 9  | 0.45                           | 0.26, 0.66 | 13                                     | 0.65                           | 0.43, 0.82 | -0.20                           | -0.46, 0.10         |
|   |  | 7.28 (3.36, 15.74)             | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

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

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result 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>Not applicable.

<sup>i</sup>Matrix tested by the independent laboratory.

**Table 7. SIMUL-qPCR *Salmonella* Assay vs. Reference Method Results for Raw Ground Beef and Raw Beef Trim – Pooled lysates (1)**

| Matrix                                    | Strain  | MPN <sup>a</sup> /test portion | N <sup>b</sup> | SIMUL-qPCR <i>Salmonella</i> Results Confirmed |                               |            | Reference Method Results (MLG 4.09) |                               |            | dPOD <sub>c</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
|---|---|--------------------------------|----------------|--|-------------------------------|------------|-------------------------------------|-------------------------------|------------|--------------------------------|---------------------|
|   |   |                                |                | x <sup>c</sup>                                 | POD <sub>c</sub> <sup>d</sup> | 95% CI     | x                                   | POD <sub>R</sub> <sup>e</sup> | 95% CI     |                                |                     |
| Ground beef<br>375 g<br>12 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211) | N/A <sup>h</sup>               | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 1.63 (1.09, 2.90)              | 20             | 8  | 0.40                          | 0.22, 0.61 | 14                                  | 0.70                          | 0.48, 0.85 | -0.30                          | -0.54, 0.01         |
|   |   | 9.26 (3.80, 22.6)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Ground beef <sup>f</sup><br>375 g<br>12 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211) | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 0.45 (0.23, 0.76)              | 20             | 4  | 0.20                          | 0.08, 0.42 | 5                                   | 0.25                          | 0.11, 0.47 | -0.05                          | -0.30, 0.21         |
|   |   | 4.46 (2.13, 9.31)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Beef trim<br>375 g<br>12 h                | <i>S. Infantis</i> , C <sub>1</sub> (SGSC 2483) | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 0.72 (0.43, 1.17)              | 20             | 11   | 0.55                          | 0.34, 0.74 | 13                                  | 0.65                          | 0.43, 0.82 | -0.10                          | -0.37, 0.19         |
|   |   | 7.28 (3.36, 15.7)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 3                                   | 0.60                          | 0.23, 0.88 | 0.40                           | -0.12, 0.77         |
| Ground beef<br>375 g<br>18 h              | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211) | N/A <sup>h</sup>               | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 1.63 (1.09, 2.90)              | 20             | 11   | 0.55                          | 0.34, 0.74 | 14                                  | 0.70                          | 0.48, 0.85 | -0.15                          | -0.41, 0.14         |
|   |   | 9.26 (3.80, 22.6)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Ground beef <sup>f</sup><br>375 g<br>18 h | <i>S. Typhimurium</i> , B<br>(USDA ERRC B-4211) | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 0.45 (0.23, 0.76)              | 20             | 4  | 0.20                          | 0.08, 0.42 | 5                                   | 0.25                          | 0.11, 0.47 | -0.05                          | -0.30, 0.21         |
|   |   | 4.46 (2.13, 9.31)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 5                                   | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Beef trim<br>375 g<br>18 h                | <i>S. Infantis</i> , C <sub>1</sub> (SGSC 2483) | N/A                            | 5              | 0  | 0.00                          | 0.00, 0.43 | 0                                   | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 0.72 (0.43, 1.17)              | 20             | 9  | 0.45                          | 0.26, 0.66 | 13                                  | 0.65                          | 0.43, 0.82 | -0.20                          | -0.46, 0.10         |
|   |   | 7.28 (3.36, 15.7)              | 5              | 5  | 1.00                          | 0.57, 1.00 | 3                                   | 0.60                          | 0.23, 0.88 | 0.40                           | -0.12, 0.77         |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

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

<sup>f</sup>dPOD<sub>c</sub> = Difference between the candidate method result and reference method result 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>Not applicable.

<sup>f</sup>Matrix tested by the independent laboratory.

Table 8. SIMUL-qPCR *Salmonella* Presumptive vs. Confirmed Results – Individual samples (1)

| Matrix                               | Strain   | MPN <sup>a</sup> /test portion     | N <sup>b</sup> | SIMUL-qPCR <i>Salmonella</i> presumptive |                                |            | SIMUL-qPCR <i>Salmonella</i> confirmed |                                |            |                                 |                     |
|--------------------------------------|--|------------------------------------|----------------|--|--------------------------------|------------|--|--------------------------------|------------|---------------------------------|---------------------|
|                                      |  |                                    |                | x <sup>c</sup>                           | POD <sub>CP</sub> <sup>d</sup> | 95% CI     | x                                      | POD <sub>CC</sub> <sup>e</sup> | 95% CI     | dPOD <sub>CP</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
| Raw ground poultry<br>375 g          | <i>S. Oranienburg</i> , C <sub>1</sub> (BEI NR-171)                      | N/A <sup>h</sup>                   | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | 1.00 (0.63, 1.63)                  | 20             | 12                                       | 0.60                           | 0.39, 0.78 | 13                                     | 0.65                           | 0.43, 0.82 | -0.05                           | -0.32, 0.23         |
|                                      |  | 9.26 (3.80, 22.5)                  | 5              | 4  | 0.80                           | 0.38, 1.00 | 4                                      | 0.80                           | 0.38, 1.00 | 0.00                            | -0.47, 0.47         |
| RTE cooked poultry<br>375 g          | <i>S. Weltevredon</i> , E <sub>1</sub> (SGSC 4929)                       | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | 1.18 (1.05, 2.05)                  | 20             | 12                                       | 0.60                           | 0.39, 0.78 | 13                                     | 0.65                           | 0.43, 0.82 | -0.05                           | -0.32, 0.23         |
|                                      |  | 9.37 (4.42, 1.00E+12) <sup>i</sup> | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Dry pet food<br>375 g                | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                              | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | 1.40 (0.91, 2.40)                  | 20             | 15                                       | 0.75                           | 0.53, 0.89 | 15                                     | 0.75                           | 0.53, 0.89 | 0.00                            | -0.26, 0.26         |
|                                      |  | 6.45 (2.88, 14.5)                  | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Dry pet food <sup>j</sup><br>375 g   | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                              | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | 0.54 (0.29, 0.91)                  | 20             | 9  | 0.45                           | 0.26, 0.66 | 9                                      | 0.45                           | 0.26, 0.66 | 0.00                            | -0.13, 0.13         |
|                                      |  | 9.37 (5.07, 1.00E+12) <sup>i</sup> | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Frankfurter<br>25 g                  | <i>S. Muenchen</i> , C <sub>2</sub> (SGSC 2490)                          | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | 1.07 (0.65, 1.76)                  | 20             | 18                                       | 0.90                           | 0.70, 0.97 | 15                                     | 0.75                           | 0.53, 0.89 | 0.15                            | -0.09, 0.38         |
|                                      |  | 4.92 (2.27, 10.7)                  | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Peanut butter<br>25 g                | <i>S. Senftenberg</i> , E <sub>4</sub> (SGSC 2516)                       | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | 1.52 (0.99, 2.35)                  | 20             | 15                                       | 0.75                           | 0.53, 0.89 | 15                                     | 0.75                           | 0.53, 0.89 | 0.00                            | -0.26, 0.26         |
|                                      |  | 9.37 (5.07, 1.00E12) <sup>i</sup>  | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Chicken carcass<br>rinsate           | <i>S. Enteritidis</i> , D <sub>1</sub> (ATCC 13076)                      | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | N/A                                | 20             | 6  | 0.30                           | 0.15, 0.52 | 6                                      | 0.30                           | 0.15, 0.52 | 0.00                            | -0.27, 0.27         |
|                                      |  | N/A                                | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Liquid Eggs<br>100 g                 | <i>S. Maregregoso</i> , 66 (SGSC 2578)                                   | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | 0.90 (0.54, 1.52)                  | 20             | 9  | 0.45                           | 0.26, 0.66 | 12                                     | 0.60                           | 0.39, 0.78 | -0.15                           | -0.41, 0.15         |
|                                      |  | 4.56 (2.21, 9.41)                  | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Stainless steel<br>1"x1"<br>AFD swab | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C. <i>freundii</i> (ATCC 43864) | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | N/A                                | 20             | 7  | 0.35                           | 0.18, 0.57 | 7                                      | 0.35                           | 0.18, 0.57 | 0.00                            | -0.28, 0.28         |
|                                      |  | N/A                                | 5              | 5  | 1.00                           | 0.57, 1.00 | 5                                      | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Stainless steel<br>4"x4 "<br>Sponge  | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C. <i>freundii</i> (ATCC 43864) | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|                                      |  | N/A                                | 20             | 8  | 0.40                           | 0.22, 0.61 | 8                                      | 0.40                           | 0.22, 0.61 | 0.00                            | -0.28, 0.28         |
|                                      |  | N/A                                | 5              | 4  | 0.80                           | 0.38, 1.00 | 4                                      | 0.80                           | 0.38, 1.00 | 0.00                            | -0.47, 0.47         |
| Stainless steel <sup>l</sup>         | <i>S. Heidelberg</i> , B   | N/A                                | 5              | 0  | 0.00                           | 0.00, 0.43 | 0                                      | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |

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|  |  |     |    |    |      |            |    |      |            |      |             |
|--|--|-----|----|----|------|------------|----|------|------------|------|-------------|
| 4" x 4"<br>AFD swab                    | (SGSC 2480)/10X C.<br><i>freundii</i> (ATCC 43864) | N/A | 20 | 14 | 0.70 | 0.48, 0.85 | 14 | 0.70 | 0.48, 0.85 | 0.00 | -0.13, 0.13 |
|  |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00 | -0.43, 0.43 |
| Plastic<br>1" x 1"<br>AFD swab         | S. Montevideo, C <sub>1</sub> (ATCC 8387)          | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00 | -0.43, 0.43 |
|  |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 4  | 0.20 | 0.08, 0.42 | 0.00 | -0.25, 0.25 |
|  |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00 | -0.43, 0.43 |
| Rubber<br>1" x 1"<br>AFD swab          | S. Panama, D <sub>1</sub> (SGSC 3583)              | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00 | -0.43, 0.43 |
|  |  | N/A | 20 | 5  | 0.25 | 0.11, 0.47 | 5  | 0.25 | 0.11, 0.47 | 0.00 | -0.26, 0.26 |
|  |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.20 | -0.28, 0.62 |
| Ceramic tile<br>1" x 1"<br>AFD swab    | S. Poona, G <sub>1</sub> (SGSC 3583)               | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00 | -0.43, 0.43 |
|  |  | N/A | 20 | 15 | 0.75 | 0.53, 0.89 | 14 | 0.70 | 0.48, 0.85 | 0.05 | -0.22, 0.31 |
|  |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00 | -0.43, 0.43 |
| Sealed concrete<br>1" x 1"<br>AFD swab | S. Anatum, E <sub>1</sub> (SGSC 2459)              | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00 | -0.43, 0.43 |
|  |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 4  | 0.20 | 0.08, 0.42 | 0.00 | -0.25, 0.25 |
|  |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.20 | -0.28, 0.62 |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

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

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result 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>Not applicable.

<sup>i</sup>All test portions in the MPN were positive, leading to the high value.

<sup>j</sup>Matrix tested by the independent laboratory.

Table 9. SIMUL-qPCR *Salmonella* Assay vs. MLG 4.09 and BAM Ch. 5 – Individual samples (1)

| Matrix  | Strain  | MPN <sup>g</sup> /test portion     | SIMUL-qPCR <i>Salmonella</i> results |                |                               |            | Reference method results <sup>e</sup> |                               |            |                                |                     |
|---|---|------------------------------------|--------------------------------------|----------------|-------------------------------|------------|---------------------------------------|-------------------------------|------------|--------------------------------|---------------------|
|   |   |                                    | N <sup>b</sup>                       | x <sup>c</sup> | POD <sub>C</sub> <sup>d</sup> | 95% CI     | x                                     | POD <sub>R</sub> <sup>f</sup> | 95% CI     | dPOD <sub>C</sub> <sup>g</sup> | 95% CI <sup>h</sup> |
| Raw ground poultry<br>375 g                         | <i>S. Oranienburg</i> , C <sub>1</sub> (BEI NR-171)                         | N/A <sup>i</sup>                   | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 1.00 (0.63, 1.63)                  | 20                                   | 12             | 0.60                          | 0.39, 0.78 | 15                                    | 0.75                          | 0.53, 0.89 | -0.15                          | -0.40, 0.13         |
|   |   | 9.26 (3.80, 22.5)                  | 5                                    | 4              | 0.80                          | 0.38, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | -0.20                          | -0.62, 0.28         |
| RTE cooked poultry<br>375 g                         | <i>S. Weltevredon</i> , E <sub>1</sub> (SGSC 4929)                          | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 1.18 (1.05, 2.05)                  | 20                                   | 12             | 0.60                          | 0.39, 0.78 | 14                                    | 0.70                          | 0.48, 0.85 | -0.10                          | -0.36, 0.18         |
|   |   | 9.37 (4.42, 1.00E+12) <sup>y</sup> | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Dry pet food<br>375 g                               | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                                 | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 1.40 (0.91, 2.40)                  | 20                                   | 15             | 0.75                          | 0.53, 0.89 | 17                                    | 0.85                          | 0.64, 0.95 | -0.10                          | -0.34, 0.15         |
|   |   | 6.45 (2.88, 14.5)                  | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Dry pet food <sup>k</sup><br>375 g                  | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                                 | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 0.54 (0.29, 0.91)                  | 20                                   | 9              | 0.45                          | 0.26, 0.66 | 10                                    | 0.50                          | 0.30, 0.70 | -0.05                          | -0.33, 0.24         |
|   |   | 9.37 (5.07, 1.00E+12) <sup>y</sup> | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Frankfurter<br>25 g                                 | <i>S. Muenchen</i> , C <sub>2</sub> (SGSC 2490)                             | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 1.07 (0.65, 1.76)                  | 20                                   | 15             | 0.75                          | 0.53, 0.89 | 12                                    | 0.60                          | 0.39, 0.78 | 0.15                           | -0.13, 0.40         |
|   |   | 4.92 (2.27, 10.7)                  | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Peanut butter<br>25 g                               | <i>S. Senftenberg</i> , E <sub>4</sub> (SGSC 2516)                          | N/A                                | 5                                    | 0              | 0.00                          | 0.0, 0.43  | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 1.52 (0.99, 2.35)                  | 20                                   | 15             | 0.75                          | 0.53, 0.89 | 19                                    | 0.95                          | 0.76, 1.00 | -0.20                          | -0.42, 0.03         |
|   |   | 9.37 (5.07, 1.00E+12) <sup>y</sup> | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Chicken carcass<br>rinsate                          | <i>S. Enteritidis</i> , D <sub>1</sub> (ATCC 13076)                         | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | N/A                                | 20                                   | 6              | 0.30                          | 0.15, 0.52 | 9                                     | 0.45                          | 0.26, 0.66 | -0.15                          | -0.41, 0.15         |
|   |   | N/A                                | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Liquids Eggs<br>100 g                               | <i>S. Marengrosso</i> , 66 (SGSC 2578)                                      | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | 0.90 (0.54, 1.52)                  | 20                                   | 9              | 0.45                          | 0.26, 0.66 | 12                                    | 0.60                          | 0.39, 0.78 | -0.15                          | -0.54, 0.01         |
|   |   | 4.56 (2.21, 9.41)                  | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Stainless steel<br>1" x 1"<br>AFD swab              | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C.<br><i>freundii</i> (ATCC 43864) | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | N/A                                | 20                                   | 7              | 0.35                          | 0.18, 0.57 | 10                                    | 0.50                          | 0.30, 0.70 | -0.15                          | -0.41, 0.15         |
|   |   | N/A                                | 5                                    | 5              | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Stainless steel<br>4" x 4"<br>Sponge                | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C.<br><i>freundii</i> (ATCC 43864) | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | N/A                                | 20                                   | 8              | 0.40                          | 0.22, 0.61 | 10                                    | 0.50                          | 0.30, 0.70 | -0.10                          | -0.37, 0.19         |
|   |   | N/A                                | 5                                    | 4              | 0.80                          | 0.38, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | -0.20                          | -0.62, 0.28         |
| Stainless steel <sup>k</sup><br>4" x 4"<br>AFD swab | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C.<br><i>freundii</i> (ATCC 43864) | N/A                                | 5                                    | 0              | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |   | N/A                                | 20                                   | 14             | 0.70                          | 0.48, 0.85 | 12                                    | 0.60                          | 0.39, 0.78 | 0.10                           | -0.18, 0.36         |



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|                                       |  |     |    |    |      |            |    |      |            |       |             |
|---------------------------------------|--|-----|----|----|------|------------|----|------|------------|-------|-------------|
|                                       |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00  | -0.43, 0.43 |
| Plastic<br>1"x 1"<br>AFD swab         | S. Montevideo, C <sub>1</sub> (ATCC<br>8387) | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 5  | 0.25 | 0.11, 0.47 | -0.05 | -0.30, 0.21 |
|                                       |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00  | -0.43, 0.43 |
| Rubber<br>1"x 1"<br>AFD swab          | S. Panama, D <sub>1</sub> (SGSC<br>3583)     | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 5  | 0.25 | 0.11, 0.47 | 9  | 0.45 | 0.26, 0.66 | -0.20 | -0.45, 0.09 |
|                                       |  | N/A | 5  | 4  | 0.80 | 0.38, 1.00 | 5  | 1.00 | 0.57, 1.00 | -0.20 | -0.62, 0.28 |
| Ceramic tile<br>1"x 1"<br>AFD swab    | S. Poona, G <sub>1</sub> (SGSC 3583)         | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 14 | 0.70 | 0.48, 0.85 | 11 | 0.55 | 0.34, 0.74 | 0.15  | -0.14, 0.41 |
|                                       |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.20  | -0.28, 0.62 |
| Sealed concrete<br>1"x 1"<br>AFD swab | S. Anatum, E <sub>1</sub> (SGSC<br>2459)     | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 5  | 0.25 | 0.11, 0.47 | -0.05 | -0.30, 0.21 |
|                                       |  | N/A | 5  | 4  | 0.80 | 0.38, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.00  | -0.47, 0.47 |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

<sup>e</sup>Reference method = MLG 4.09 for raw ground poultry, RTE cooked poultry, frankfurter, chicken carcass rinsate, pasteurized liquid egg; FDA BAM Ch. 5 for dry pet food, peanut butter, environmental surfaces.

<sup>f</sup>POD<sub>r</sub> = Reference method positive outcomes divided by the total number of trials.

<sup>g</sup>dPOD<sub>c</sub> = Difference between the candidate method result and reference method result POD values.

<sup>h</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>i</sup>Not applicable.

<sup>j</sup>All test portions in the MPN were positive, leading to the high value.

<sup>k</sup>Matrix tested by the independent laboratory.

Table 10. SIMUL-qPCR *Salmonella* Assay Presumptive vs. Confirmed Results – Pooled lysates (1)

| Matrix  | Strain   | MPN <sup>g</sup> /test portion     | N <sup>b</sup> | SIMUL-qPCR <i>Salmonella</i> Presumptive Pooled |                                |            | SIMUL-qPCR <i>Salmonella</i> confirmed Single |                                |            | dPOD <sub>CF</sub> <sup>f</sup> | 95% CI <sup>g</sup> |
|---|--|------------------------------------|----------------|---|--------------------------------|------------|---|--------------------------------|------------|---------------------------------|---------------------|
|   |  |                                    |                | x <sup>c</sup>                                  | POD <sub>CF</sub> <sup>d</sup> | 95% CI     | x   | POD <sub>CC</sub> <sup>e</sup> | 95% CI     |                                 |                     |
| Raw ground poultry<br>375 g                         | <i>S. Oranienburg</i> , C <sub>1</sub> (BEI NR-171)                      | N/A <sup>h</sup>                   | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.00 (0.63, 1.63)                  | 20             | 13  | 0.65                           | 0.43, 0.82 | 13  | 0.65                           | 0.43, 0.82 | 0.00                            | -0.28, 0.28         |
|   |  | 9.26 (3.80, 22.5)                  | 5              | 4   | 0.80                           | 0.38, 1.00 | 4   | 0.80                           | 0.38, 1.00 | 0.00                            | -0.47, 0.47         |
| RTE cooked poultry<br>375 g                         | <i>S. Weltevredon</i> , E <sub>1</sub> (SGSC 4929)                       | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.18 (1.05, 2.05)                  | 20             | 12  | 0.60                           | 0.39, 0.78 | 13  | 0.65                           | 0.43, 0.82 | -0.05                           | -0.32, 0.23         |
|   |  | 9.37 (4.42, 1.00E+12) <sup>y</sup> | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Dry pet food<br>375 g                               | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                              | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.40 (0.91, 2.40)                  | 20             | 13  | 0.65                           | 0.43, 0.82 | 15  | 0.75                           | 0.53, 0.89 | -0.10                           | -0.36, 0.18         |
|   |  | 6.45 (2.88, 14.5)                  | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Dry pet food <sup>i</sup><br>375 g                  | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                              | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 0.54 (0.29, 0.91)                  | 20             | 9   | 0.45                           | 0.26, 0.66 | 9   | 0.45                           | 0.26, 0.66 | 0.00                            | -0.13, 0.13         |
|   |  | 9.37 (5.07, 1.00E+12) <sup>y</sup> | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Frankfurter<br>25 g                                 | <i>S. Muenchen</i> , C <sub>2</sub> (SGSC 2490)                          | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.07 (0.65, 1.76)                  | 20             | 15  | 0.75                           | 0.53, 0.89 | 15  | 0.75                           | 0.53, 0.89 | 0.00                            | -0.26, 0.26         |
|   |  | 4.92 (2.27, 10.7)                  | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Peanut butter<br>25 g                               | <i>S. Senftenberg</i> , E <sub>4</sub> (SGSC 2516)                       | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | 1.52 (0.99, 2.35)                  | 20             | 15  | 0.75                           | 0.53, 0.89 | 15  | 0.75                           | 0.53, 0.89 | 0.00                            | -0.26, 0.26         |
|   |  | 9.37 (5.07, 1.00E+12) <sup>y</sup> | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Chicken carcass<br>rinsate                          | <i>S. Enteritidis</i> , D <sub>1</sub> (ATCC 13076)                      | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | N/A                                | 20             | 6   | 0.30                           | 0.15, 0.52 | 6   | 0.30                           | 0.15, 0.52 | 0.00                            | -0.27, 0.27         |
|   |  | N/A                                | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Stainless steel<br>1" x 1"<br>AFD swab              | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C. <i>freundii</i> (ATCC 43864) | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | N/A                                | 20             | 7   | 0.35                           | 0.18, 0.57 | 7   | 0.35                           | 0.18, 0.57 | 0.00                            | -0.28, 0.28         |
|   |  | N/A                                | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Stainless steel<br>4" x 4"<br>Sponge                | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C. <i>freundii</i> (ATCC 43864) | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | N/A                                | 20             | 9   | 0.45                           | 0.26, 0.66 | 8   | 0.40                           | 0.22, 0.61 | 0.05                            | -0.24, 0.33         |
|   |  | N/A                                | 5              | 4   | 0.80                           | 0.38, 1.00 | 4   | 0.80                           | 0.38, 1.00 | 0.00                            | -0.47, 0.47         |
| Stainless steel <sup>j</sup><br>4" x 4"<br>AFD swab | <i>S. Heidelberg</i> , B (SGSC 2480)/10X C. <i>freundii</i> (ATCC 43864) | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |
|   |  | N/A                                | 20             | 13  | 0.65                           | 0.43, 0.82 | 14  | 0.70                           | 0.48, 0.85 | -0.05                           | -0.21, 0.11         |
|   |  | N/A                                | 5              | 5   | 1.00                           | 0.57, 1.00 | 5   | 1.00                           | 0.57, 1.00 | 0.00                            | -0.43, 0.43         |
| Plastic   | <i>S. Montevideo</i> , C <sub>1</sub> (ATCC                              | N/A                                | 5              | 0   | 0.00                           | 0.00, 0.43 | 0   | 0.00                           | 0.00, 0.43 | 0.00                            | -0.43, 0.43         |

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|  |  |     |    |    |      |            |    |      |            |       |             |
|--|--|-----|----|----|------|------------|----|------|------------|-------|-------------|
| 1" x 1"<br>AFD swab                    | 8387)                                    | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 4  | 0.20 | 0.08, 0.42 | 0.00  | -0.25, 0.25 |
|  |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00  | -0.43, 0.43 |
| Rubber<br>1" x 1"<br>AFD swab          | S. Panama, D <sub>1</sub> (SGSC<br>3583) | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|  |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 5  | 0.25 | 0.11, 0.47 | -0.05 | -0.30, 0.21 |
|  |  | N/A | 5  | 4  | 0.80 | 0.38, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.00  | -0.47, 0.47 |
| Ceramic tile<br>1" x 1"<br>AFD swab    | S. Poona, G <sub>1</sub> (SGSC 3583)     | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|  |  | N/A | 20 | 14 | 0.70 | 0.48, 0.85 | 14 | 0.70 | 0.48, 0.85 | 0.00  | -0.27, 0.27 |
|  |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00  | -0.43, 0.43 |
| Sealed concrete<br>1" x 1"<br>AFD swab | S. Anatum, E <sub>1</sub> (SGSC<br>2459) | N/A | 5  | 0  | 0.00 | 0.00-0.43  | 0  | 0.00 | 0.00-0.43  | 0.00  | -0.43, 0.43 |
|  |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 4  | 0.20 | 0.08, 0.42 | 0.00  | -0.25, 0.25 |
|  |  | N/A | 5  | 4  | 0.80 | 0.38, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.00  | -0.47, 0.47 |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

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

<sup>f</sup>dPOD<sub>CP</sub> = Difference between the candidate method presumptive result and candidate method confirmed result 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>Not applicable.

<sup>i</sup>All test portions in the MPN were positive, leading to the high value.

<sup>j</sup>Matrix tested by the independent laboratory.

Table 11. SIMUL-qPCR *Salmonella* Assay vs. Reference Method Results – Pooled lysates (1)

| Matrix  | Strain   | MPN <sup>a</sup> /test portion      | N <sup>b</sup> | SIMUL-qPCR <i>Salmonella</i> pooled results |                               |            | Reference method results <sup>e</sup> |                               |            | dPOD <sub>c</sub> <sup>g</sup> | 95% CI <sup>h</sup> |
|---|--|-------------------------------------|----------------|---|-------------------------------|------------|---------------------------------------|-------------------------------|------------|--------------------------------|---------------------|
|   |  |                                     |                | x <sup>c</sup>                              | POD <sub>c</sub> <sup>d</sup> | 95% CI     | x                                     | POD <sub>R</sub> <sup>f</sup> | 95% CI     |                                |                     |
| Raw ground poultry<br>375 g                         | <i>S. Oranienburg</i> , C <sub>1</sub> (BEI NR-171)                      | N/A <sup>i</sup>                    | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 1.00 (0.63, 1.63)                   | 20             | 13  | 0.65                          | 0.43, 0.82 | 15                                    | 0.75                          | 0.53, 0.89 | -0.10                          | -0.36, 0.18         |
|   |  | 9.26 (3.80, 22.5)                   | 5              | 4   | 0.80                          | 0.38, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | -0.20                          | -0.62, 0.28         |
| RTE cooked poultry<br>375 g                         | <i>S. Weltevredon</i> , E <sub>1</sub> (SGSC 4929)                       | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 1.18 (1.05, 2.05)                   | 20             | 13  | 0.65                          | 0.43, 0.82 | 14                                    | 0.70                          | 0.48, 0.85 | -0.05                          | -0.32, 0.23         |
|   |  | 9.37 (4.42, 1.00E+12) <sup>j</sup>  | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Dry pet food<br>375 g                               | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                              | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 1.40 (0.91, 2.40)                   | 20             | 15  | 0.75                          | 0.53, 0.89 | 17                                    | 0.85                          | 0.64, 0.95 | -0.10                          | -0.34, 0.15         |
|   |  | 6.45 (2.88, 14.5)                   | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Dry pet food <sup>k</sup><br>375 g                  | <i>S. Schwarzengrund</i> , B (BEI NR-28796)                              | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 0.54 (0.29, 0.91)                   | 20             | 9   | 0.45                          | 0.26, 0.66 | 10                                    | 0.50                          | 0.30, 0.70 | -0.05                          | -0.33, 0.24         |
|   |  | 9.37E (5.07, 1.00E+12) <sup>j</sup> | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Frankfurter<br>25 g                                 | <i>S. Muenchen</i> , C <sub>2</sub> (SGSC 2490)                          | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 1.07 (0.65, 1.76)                   | 20             | 15  | 0.75                          | 0.53, 0.89 | 12                                    | 0.60                          | 0.39, 0.78 | 0.15                           | -0.13, 0.40         |
|   |  | 4.92 (2.27, 10.7)                   | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Peanut butter<br>25 g                               | <i>S. Senftenberg</i> , E <sub>4</sub> (SGSC 2516)                       | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | 1.52 (0.99, 2.35)                   | 20             | 15  | 0.75                          | 0.53, 0.89 | 19                                    | 0.95                          | 0.76, 1.00 | -0.20                          | -0.42, 0.03         |
|   |  | 9.37 (5.07, 1.00E+12) <sup>j</sup>  | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Chicken carcass<br>rinsate                          | <i>S. Enteritidis</i> , D <sub>1</sub> (ATCC 13076)                      | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | N/A                                 | 20             | 6   | 0.30                          | 0.15, 0.52 | 9                                     | 0.45                          | 0.26, 0.66 | -0.15                          | -0.41, 0.14         |
|   |  | N/A                                 | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Stainless steel<br>1" x 1"<br>AFD swab              | <i>S. Heidelberg</i> , B (SGSC 2480)/10X <i>C. freundii</i> (ATCC 43864) | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | N/A                                 | 20             | 7   | 0.35                          | 0.18, 0.57 | 10                                    | 0.50                          | 0.30, 0.70 | -0.15                          | -0.41, 0.15         |
|   |  | N/A                                 | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Stainless steel <sup>k</sup><br>4" x 4"<br>AFD swab | <i>S. Heidelberg</i> , B (SGSC 2480)/10X <i>C. freundii</i> (ATCC 43864) | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | N/A                                 | 20             | 13  | 0.65                          | 0.43, 0.82 | 12                                    | 0.60                          | 0.39, 0.78 | 0.05                           | -0.23, 0.32         |
|   |  | N/A                                 | 5              | 5   | 1.00                          | 0.57, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | 0.00                           | -0.43, 0.43         |
| Stainless steel<br>4" x 4"<br>Sponge                | <i>S. Heidelberg</i> , B (SGSC 2480)/10X <i>C. freundii</i> (ATCC 43864) | N/A                                 | 5              | 0   | 0.00                          | 0.00, 0.43 | 0                                     | 0.00                          | 0.00, 0.43 | 0.00                           | -0.43, 0.43         |
|   |  | N/A                                 | 20             | 8   | 0.40                          | 0.22, 0.61 | 10                                    | 0.50                          | 0.30, 0.70 | -0.10                          | -0.37, 0.19         |
|   |  | N/A                                 | 5              | 4   | 0.80                          | 0.38, 1.00 | 5                                     | 1.00                          | 0.57, 1.00 | -0.20                          | -0.62, 0.28         |

|                                       |  |     |    |    |      |            |    |      |            |       |             |
|---------------------------------------|--|-----|----|----|------|------------|----|------|------------|-------|-------------|
| Plastic<br>1"x 1"<br>AFD swab         | S. Montevideo, C <sub>1</sub> (ATCC<br>8387) | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 5  | 0.25 | 0.11, 0.47 | -0.05 | -0.30, 0.21 |
|                                       |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 5  | 1.00 | 0.57, 1.00 | 0.00  | -0.43, 0.43 |
| Rubber<br>1"x 1"<br>AFD swab          | S. Panama, D <sub>1</sub> (SGSC<br>3583)     | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 5  | 0.25 | 0.11, 0.47 | 9  | 0.45 | 0.26, 0.66 | -0.20 | -0.45, 0.09 |
|                                       |  | N/A | 5  | 4  | 0.80 | 0.38, 1.00 | 5  | 1.00 | 0.57, 1.00 | -0.20 | -0.62, 0.09 |
| Ceramic tile<br>1"x 1"<br>AFD swab    | S. Poona, G <sub>1</sub> (SGSC 3583)         | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 14 | 0.70 | 0.48, 0.85 | 11 | 0.55 | 0.34, 0.74 | 0.15  | -0.14, 0.41 |
|                                       |  | N/A | 5  | 5  | 1.00 | 0.57, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.20  | -0.28, 0.62 |
| Sealed concrete<br>1"x 1"<br>AFD swab | S. Anatum, E <sub>1</sub> (SGSC<br>2459)     | N/A | 5  | 0  | 0.00 | 0.00, 0.43 | 0  | 0.00 | 0.00, 0.43 | 0.00  | -0.43, 0.43 |
|                                       |  | N/A | 20 | 4  | 0.20 | 0.08, 0.42 | 5  | 0.25 | 0.11, 0.47 | -0.05 | -0.30, 0.21 |
|                                       |  | N/A | 5  | 4  | 0.80 | 0.38, 1.00 | 4  | 0.80 | 0.38, 1.00 | 0.00  | -0.47, 0.47 |

<sup>a</sup>MPN = Most Probable Number is based on the POD of reference method test portions using the LCF MPN calculator, with 95% confidence interval.

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

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

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

<sup>e</sup>Reference method = MLG 4.09 for raw ground poultry, RTE cooked poultry, frankfurter, chicken carcass rinsate, pasteurized liquid egg; FDA BAM Ch. 5 for dry pet food, peanut butter, environmental surfaces.

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

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

<sup>h</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>i</sup>Not applicable.

<sup>j</sup>All test portions in the MPN were positive, leading to the high value.

<sup>k</sup>Matrix tested by the independent laboratory.

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