SALMONELLA ENUMERATION METHOD COMPARISON IN ONION AND GARLIC STUDY REPORT

Comparison of the *Salmonella* Enumeration Methods via MPN, XLD, and RAPID' Salmonella agars in Onion and Garlic Samples

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SUMMARY

Samples of raw onion and garlic from California, USA were artificially-inoculated with *Salmonella* at approximately 2 log CFU/g and 3 log CFU/g and shipped overnight to participating laboratories for enumeration using three different methods: (i) Most Probable Number (MPN), (ii) Tryptic Soy Agar overlaid with Xylose Lysine Deoxycholate Agar (TSA/XLD), and (iii) RAPID' *Salmonella* agar by spread-plating (RAP-SAL). The data generated in this method comparison study confirmed that all three enumeration methods were useful for quantifying *Salmonella* in raw garlic and onion. Study results demonstrated equivalent performance by the three participating laboratories. The MPN method gave statistically greater recovery of low *Salmonella* populations in inoculated onion and garlic samples. For samples that were inoculated at a greater *Salmonella* population level, there was no statistical difference observed in results for MPN and TSA/XLD methods. The RAP-SAL method resulted in the lowest average population of *Salmonella* for all garlic and onion samples tested. Selectivity of TSA/XLD was lower than both MPN and RAP-SAL methods. This was due to high population levels of background microflora that were observed on TSA/XLD plates, making this method difficult to use for the purpose of detecting low population levels of *Salmonella.* Based on the limited number of samples that were evaluated among the three laboratories, the MPN method appears to be most promising in terms of recovery of *Salmonella.* This may be a very important consideration as population levels of *Salmonella* in raw garlic and raw onion are expected to be at low levels.

STUDY OVERVIEW

There are various methods available for the enumeration of *Salmonella* in food products that range across a variety of platforms, such as traditional selective agar overlayed on non-selective agar, chromogenic agars, and Most Probable Number (MPN) enrichment tube methods. Raw garlic and onion present particular challenges in the enumeration of *Salmonella* populations as they contain natural antimicrobial properties that are known to inhibit microbial growth and also have high levels of native microflora that may mask *Salmonella* populations on insufficiently inhibitory media. Recalls and outbreaks associated with raw onions in recent years highlight the need to evaluate the performance of the existing enumeration methods, which can be used to assess natural contamination levels in onions and other raw agriculture commodities such as garlic. [1,2]

PURPOSE

The purpose of this study was to determine the performance of three *Salmonella* enumeration methods based on the recovery of inoculated *Salmonella* on ASTA-member donated raw garlic and onion samples collected in the summer of 2024.

METHODS

Freshly harvested onion and garlic bulbs from California, USA were provided by an ASTA member and were kept under the refrigeration condition (5±1°C) until each inoculation trial. The average weights (avg ± SD) of onion and garlic were 96.04g ± 17.84g and 46.42g ± 10.63g, respectively.

For the preparation of inocula, *Salmonella* strains were individually inoculated into 10-ml tubes of tryptic soy broth (TSB) and incubated at 35°C for 24 hours. Each overnight *Salmonella* culture was plated on 6 tryptic soy agar (TSA) plates, followed by incubation at 35°C for 48 hours to obtain bacterial lawns. For each strain, 3 ml of phosphate buffer (PB) per plate was used to harvest cells and the culture was adjusted to be approximately 10^9 CFU/ml. Equal portions of the prepared *Salmonella* strains were combined to create a 5-strain *Salmonella* cocktail.

For preparation of inoculated onion and garlic samples, individual onion and garlic bulbs were surface-inoculated with a 5-strain *Salmonella* cocktail at 1:1000 inoculum-to-sample ratio to inoculate the product at approximately 10^3 CFU/g (for low inoculation level) and 10^4 CFU/g (for high inoculation level). The samples were inoculated at 10-fold higher level than the target level as the preliminary trials indicated an approximately 1 log reduction of the inoculum during the 24-hour adaptation phase under the refrigeration (5±1°C). A set of inoculated samples were enumerated immediately after inoculation to determine the initial inoculation level. All remaining inoculated samples were stored in a refrigerator for 2 hours to facilitate bacterial attachment.

A total of 15 replicate (5 replicates per lab) samples of inoculated onion and garlic were shipped overnight to participating food microbiology laboratories for simultaneous *Salmonella* enumerations by 3 methods: (i) Most Probable Number (MPN), (ii) Tryptic Soy Agar overlaid with Xylose Lysine Deoxycholate Agar (TSA/XLD), and (iii) RAPID' *Salmonella* agar by spread-plating (RAP-SAL). Each sample was diluted using 1 part sample to 1 part Tryptic Soy Broth (TSB) containing 0.5% K_2SO_3. The diluted samples were manually shaken vigorously for 1 minute. The TSB rinsate was then serially diluted 10-fold in PB prior to plating.

Table 1. The *Salmonella* strains used in this study.

Organism	External Reference #	Associated Product	CLCC#[1]
Salmonella Typhimurium	ATCC 13311	Feces, Human	SAL-2
Salmonella Anatum	ATCC 9270	Pork Liver	SAL-3
Salmonella Senftenberg	NA	Garlic Powder	SAL-113
Salmonella Tennessee	NA	Onion	SAL-395
Salmonella Montevideo	NA	Onion	SAL-449

[1]Certified Laboratories Culture Collection Number

Aliquots of diluted samples were plated onto TSA/XLD and RAP-SAL plates, which were incubated at 35-37°C for 24 and 48 hours. After incubation, colonies with typical *Salmonella* morphology were counted.

For MPN, 3 1-ml aliquots of TSB rinsate were added to 3 10-ml tubes of TSB. Additional 1-ml aliquots of TSB rinsate were further serially diluted in PB. Three 1-ml aliquots of each PB serial dilutions were inoculated into 3 10-ml tubes of TSB. All TSB tubes were incubated at 35-37°C for 24 hours. After incubation, 0.1 ml from each TSB tube was transferred into 10 ml Rappaport-Vassiliadis (RV) broth tubes, which were then incubated at 42°C for 24 hours. RV tubes were streaked onto RAP-SAL and incubated as described above. Typical colonies on RAP-SAL plates were considered positive for *Salmonella*, which was used to define *Salmonella*-positive MPN tubes.

In addition to the inoculated samples, three replicate samples of uninoculated onion and garlic samples were analyzed by the same respective enumeration methods and an Aerobic Plate Count was performed by each participating laboratory.

For statistical analysis, a significant difference in the means of the groups was tested using the Tukey-Kramer test at $P \leq 0.05$.

RESULTS & DISCUSSION

Detailed results for the inoculated onion and garlic samples are provided in Appendix Tables A1 to A4. Results are summarized below in Figures 1 to 4 and Tables 2 and 3. For *Salmonella* enumeration results from TSA/XLD and RAP-SAL, 24-hour counts were further compared with the MPN method as there was no statistically significant difference between the 24-hour and 48-hour counts within each plating agar method.

Immediately following inoculation, the initial *Salmonella* counts (Avg ± SD) in onion samples with low- and high-inoculation levels were determined to be 3.16 ± 0.02 and 4.06 ± 0.06 log CFU/g, respectively. The initial *Salmonella* count (Avg ± SD) in garlic samples with low- and high-inoculation levels were determined to be 2.95 ± 0.15 and 3.84 ± 0.25 log CFU/g, respectively. The initial inoculation levels were adjusted to be approximately 1 log CFU/g higher than the target levels to account for the die-off encountered during the 24-hour refrigerated adaptation phase.

Results of the enumeration study (Table 2) indicate that the MPN method, allowed for statistically greater recovery of *Salmonella* from onion and garlic samples when the inoculation level was low (2.0 log CFU/g). When the inoculation level was high (3.0 log CFU/g), there was no statistical difference observed between recovery of *Salmonella* from onion and garlic using MPN and TSA/XLD methods. The populations of *Salmonella* that were recovered using the RAP-SAL method were statistically less than populations recovered using MPN and TSA/XLD methods for 3.0 log CFU/g inoculation level of onion and 2.0 log CFU/g inoculation level of garlic. The RAP-SAL method resulted in the lowest average population of *Salmonella* for all garlic and onion samples tested.

Table 2. Summary of average *Salmonella* counts (log CFU/g ± SD or log MPN/g ± SD) recovered from inoculated onion and garlic samples using different *Salmonella* enumeration methods between three laboratories*.

Matrix	Inoculation Level	MPN	RAP-SAL	TSA/XLD
Onion	2.0 log CFU/g	2.0 ± 0.5^{a}	1.5 ± 0.4^{c}	1.6 ± 0.5^{bc}
	3.0 log CFU/g	2.9 ± 0.3^{a}	2.7 ± 0.2^{c}	2.9 ± 0.1^{ab}
Garlic	2.0 log CFU/g	2.4 ± 0.3^{a}	1.8 ± 0.3^{c}	2.2 ± 0.2^{b}
	3.0 log CFU/g	3.4 ± 0.5^{a}	3.1 ± 0.2^{b}	3.3 ± 0.3^{ab}

*The means without overlapping letters within each matrix and inoculation level are statistically different via the Tukey-Kramer test.

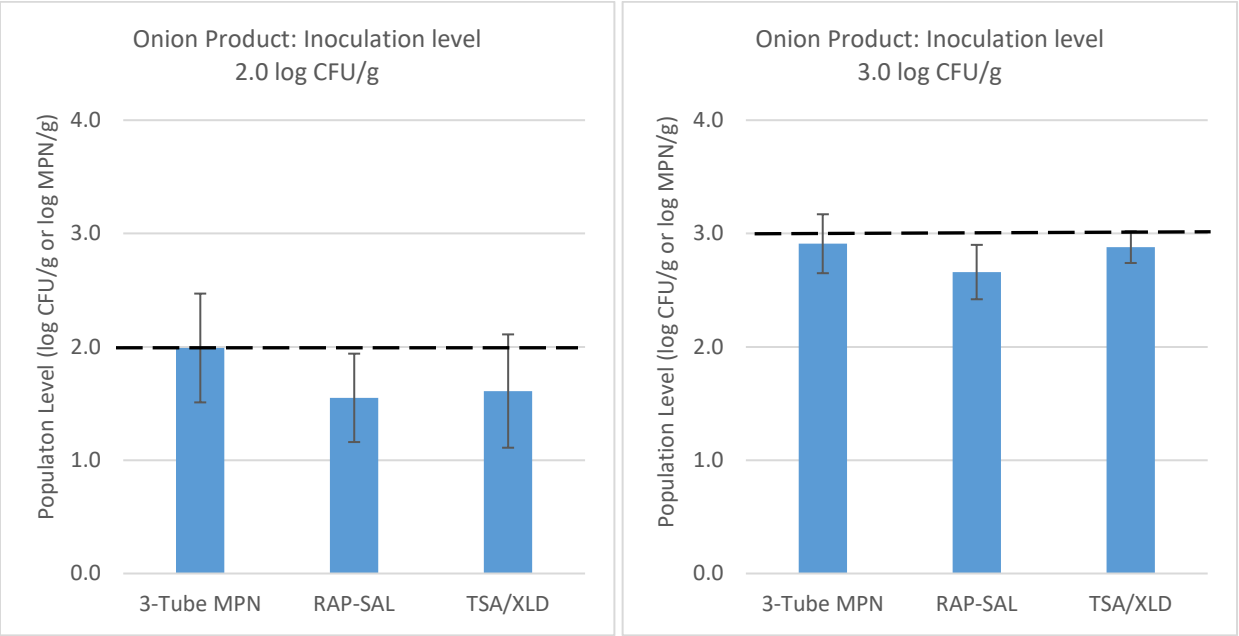

Figure 1. Comparison of *Salmonella* recovery from artificially inoculated onion product samples at 2.0 log CFU/g (left) and 3.0 log CFU/g (right) inoculation levels using three different microbiological methods. The error bars represent standard deviation (n=15). Dashed line indicates the expected inoculation level.

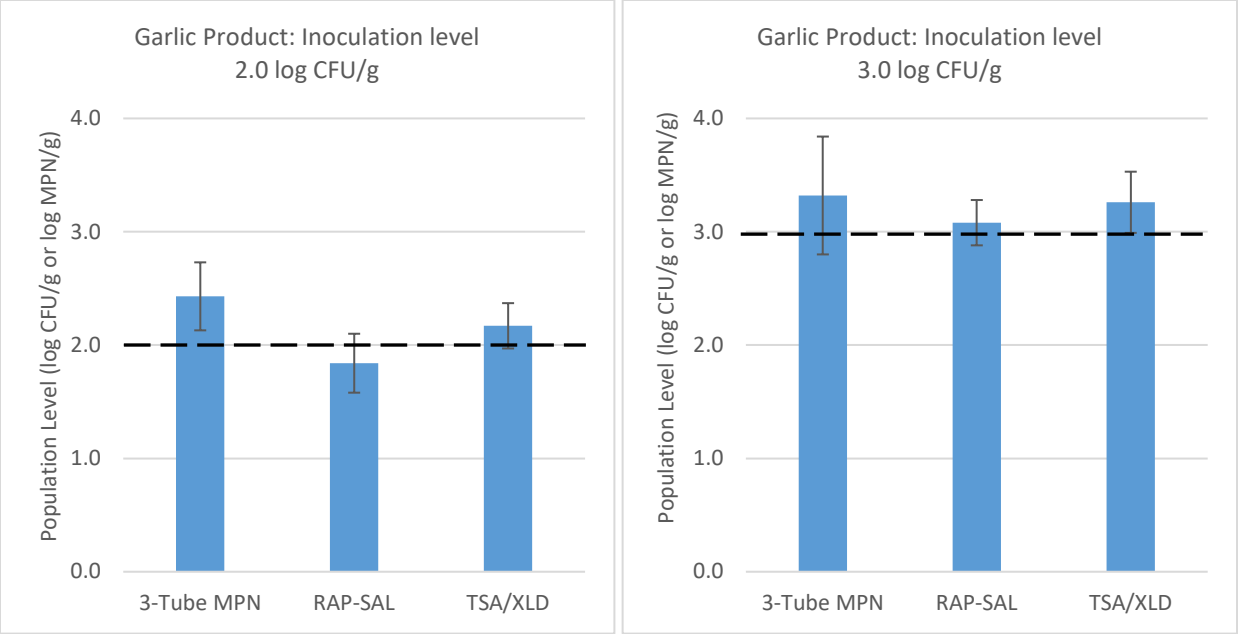

Figure 2. Comparison of *Salmonella* recovery from artificially inoculated garlic product samples at 2.0 log CFU/g (left) and 3.0 log CFU/g (right) inoculation levels using three different microbiological methods. The error bars represent standard deviation (n=15). Dashed line indicates the expected inoculation level.

Table 3 shows average data generated by the three performing laboratories. Despite the small number of samples and small number of replicate observations, there were no performance differences between the laboratories. This suggests that the methods and laboratories were performing as designed and lab-to-lab variability did not affect results.

Table 3. Comparison of *Salmonella* recovery from artificially inoculated onion and garlic samples at 2.0 log CFU/g and 3.0 log CFU/g inoculation levels between participating laboratories. Microbiological counts are reported as log CFU/g or log MPN/g.

Matrix	Inoculation Level	Analysis	Lab A	Lab B	Lab C
Onion	2.0 log CFU/g	MPN	1.9 ± 0.4	2.2 ± 0.5	1.9 ± 0.5
		RAP-SAL	1.5 ± 0.5	1.7 ± 0.4	1.5 ± 0.4
		TSA/XLD	1.7 ± 0.5	1.7 ± 0.6	1.4 ± 0.5
	3.0 log CFU/g	MPN	2.8 ± 0.3	2.9 ± 0.3	3.0 ± 0.0
		RAP-SAL	2.5 ± 0.1	2.7 ± 0.2	2.8 ± 0.4
		TSA/XLD	2.9 ± 0.2	2.9 ± 0.1	2.9 ± 0.2
Garlic	2.0 log CFU/g	MPN	2.2 ± 0.3	2.5 ± 0.2	2.6 ± 0.3
		RAP-SAL	1.9 ± 0.1	1.7 ± 0.4	1.9 ± 0.1
		TSA/XLD	2.1 ± 0.2	2.1 ± 0.1	2.3 ± 0.2
	3.0 log CFU/g	MPN	3.3 ± 0.2	3.5 ± 0.6	3.4 ± 0.8
		RAP-SAL	2.9 ± 0.1	3.1 ± 0.2	3.2 ± 0.2
		TSA/XLD	3.0 ± 0.2	3.4 ± 0.2	3.4 ± 0.2

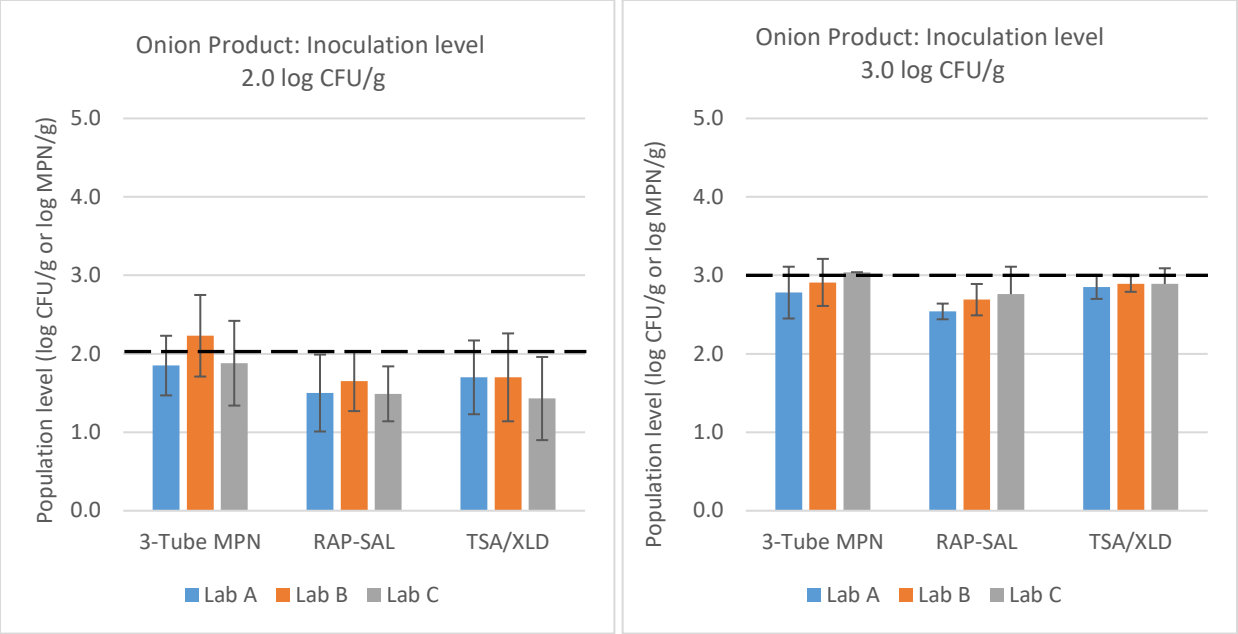

Figure 3. Comparison of *Salmonella* recovery from artificially inoculated onion samples at 2.0 log CFU/g (left) and 3.0 log CFU/g (right) inoculation levels between three laboratories using three different microbiological methods. The error bars represent standard deviation (n=5). Dashed line indicates the expected inoculation level.

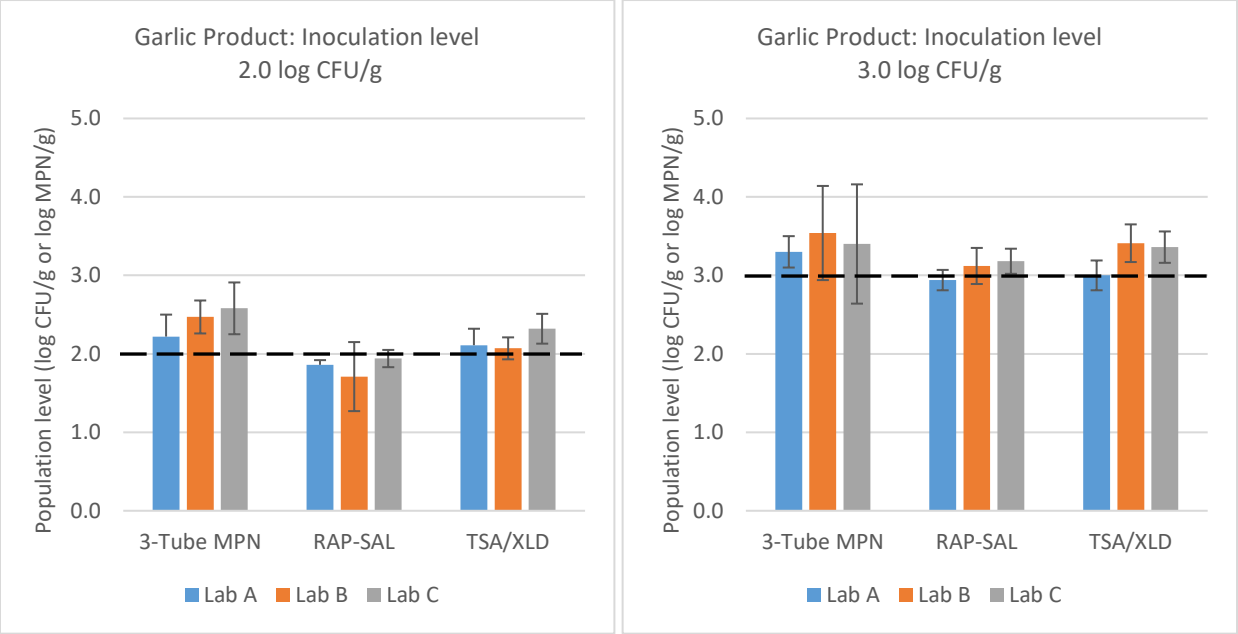

Figure 4. Comparison of *Salmonella* recovery from artificially inoculated garlic samples at 2.0 log CFU/g (left) and 3.0 log CFU/g (right) inoculation levels between three laboratories using three different microbiological methods. The error bars represent standard deviation (n=5). Dashed line indicates the expected inoculation level.

Detailed results for the uninoculated onion and garlic samples are provided in Appendix Tables A5 and A6.

The APC levels in uninoculated onions varied considerably and ranged between 2.87 to 4.75 log CFU/g. The APC levels in the uninoculated garlic samples ranged between 5.78 to >7.75 log CFU/g. *Salmonella* was not detected in any of the uninoculated garlic and onion samples (limit of detection of <0.0 log CFU/g or <-0.5 log MPN/g) that were tested from either of the three labs, however, reporting methods for the three laboratories varied (Tables A5 and A6) based on the dilution at which the absence of *Salmonella* was determined.

The presence of relatively high background microflora populations in garlic samples and onion samples could have interfered with *Salmonella* recovery, particularly on TSA/XLD, due to its inferior selectivity. This observation raises concerns about the utility of the TSA/XLD in quantifying small populations (i.e., <50 CFU/g) of *Salmonella* in raw onion and garlic in the presence of high background microflora, as competing microflora can mask the presence of *Salmonella*. While RAP-SAL yielded lower *Salmonella* counts (Table 2) compared to TSA/XLD, the background microflora was generally less pronounced on RAP-SAL, which allowed for easier identification of typical *Salmonella* colonies. Atypical colonies also were observed in garlic samples enumerated using the MPN method, however, the overall interpretation of the presence of *Salmonella* was easier due to the additional selectivity afforded by the RV enrichment step.

APPENDIX A

Table A1. Population levels of *Salmonella* recovered from artificially-inoculated onion product samples at 2.0 log CFU/g inoculum level using three different methods between three participating laboratories. Microbiological counts are reported as log CFU/g or log MPN/g. The limit of detection is 0.0 log CFU/g by the plating method -0.5 log MPN/g by the MPN method.

Analysis	Replicate	Result log CFU/g or log MPN/g		
		Lab A	Lab B	Lab C
3-Tube MPN	A	1.4	2.0	2.0
	B	1.9	1.6	1.6
	C	2.4	2.3	2.0
	D	1.6	2.2	2.7
	E	2.0	>3.0	1.2
	Average ± SD[2]	1.9 ± 0.4	2.2 ± 0.5[5]	1.9 ± 0.5
BIO-RAD Rapid *Salmonella* Agar (24 hour read)	A	0.7	1.5	1.3
	B	1.8	1.3	1.7
	C	2.0	2.2	1.7
	D	1.5	1.9	1.8
	E	1.6	1.3	1.0
	Average ± SD[2]	1.5 ± 0.5	1.7 ± 0.4	1.5 ± 0.4
BIO-RAD Rapid *Salmonella* Agar (48 hour read)	A	0.7	1.6	1.4
	B	1.8	1.4	1.7
	C	2.0	2.3	1.8
	D	1.5[3]	2.0	2.0
	E	1.6	1.5	1.1
	Average ± SD[2]	1.5 ± 0.4	1.8 ± 0.4	1.6 ± 0.4
TSA/XLD[1] (24 hour read)	A	1.4[4]	1.6	1.5
	B	2.1[4]	0.8	0.7
	C	2.3[4]	2.3	1.8
	D	1.3[4]	2.1	2.1
	E	1.4[4]	1.6	1.1
	Average ± SD[2]	1.7 ± 0.5	1.7 ± 0.6	1.4 ± 0.5
TSA/XLD[1] (48 hour read)	A	1.4[4]	1.8	1.6
	B	2.1[4]	1.1	1.9
	C	2.3[4]	2.3	1.9
	D	1.3[4]	2.1	2.1
	E	1.4[4]	1.7	1.5
	Average ± SD[2]	1.7 ± 0.5	1.8 ± 0.5	1.8 ± 0.2

[1]TSA/XLD: TSA overlaid with Xylose Lysine Deoxycholate agar; [2]Average ± SD: average ± standard deviation; [3]Data reported at 1.5 log CFU/g are typical *Salmonella* colonies; atypical colonies at TNTC were also recovered; [4]Data reported at 1.4-2.3 log CFU/g are typical for *Salmonella* colonies; atypical colonies at 2.5-4.4 log CFU/g were also recovered; [5]Value of >3.0 was set to 3.0 log MPN/g for calculation purposes.

Table A2. Population levels of *Salmonella* recovered from artificially-inoculated onion product samples at 3.0 log CFU/g inoculum level using three different methods between three participating laboratories. Microbiological counts are reported as log CFU/g or log MPN/g. The limit of detection is 0.0 log CFU/g by the plating method -0.5 log MPN/g by the MPN method.

Analysis	Replicate	Result log CFU/g or log MPN/g		
		Lab A	Lab B	Lab C
3-Tube MPN	A	2.6	>3.0	>3.0
	B	2.6	3.0	3.0
	C	2.6	>3.0	>3.0
	D	2.6	2.4	3.0
	E	3.4	>3.0	>3.0
	Average ± SD[2]	2.8 ± 0.3	2.9 ± 0.3[5]	3.0 ± 0.0[5]
BIO-RAD Rapid *Salmonella* Agar (24 hour read)	A	2.7	2.9	3.2
	B	2.5	2.4	2.9
	C	2.5	2.7	2.9
	D	2.5	2.6	2.4
	E	2.5	2.8	2.4
	Average ± SD[2]	2.5 ± 0.1	2.7 ± 0.2	2.8 ± 0.4
BIO-RAD Rapid *Salmonella* Agar (48 hour read)	A	2.7	2.9	3.3
	B	2.5[3]	2.4	3.0
	C	2.5	3.0	2.9
	D	2.5	2.8	2.9
	E	2.5[3]	2.9	2.7
	Average ± SD[2]	2.5 ± 0.1	2.8 ± 0.2	3.0 ± 0.2
TSA/XLD[1] (24 hour read)	A	3.0[4]	2.8	3.1
	B	2.6[4]	2.8	3.0
	C	2.9[4]	3.0	2.9
	D	2.9[4]	2.8	2.6
	E	2.9[4]	2.9	2.8
	Average ± SD[2]	2.9 ± 0.2	2.9 ± 0.1	2.9 ± 0.2
TSA/XLD[1] (48 hour read)	A	3.0[4]	3.1	3.5
	B	2.6[4]	2.8	3.2
	C	2.9[4]	3.0	3.1
	D	2.9[4]	2.8	2.7
	E	2.9[4]	2.9	2.9
	Average ± SD[2]	2.9 ± 0.2	2.9 ± 0.1	3.1 ± 0.3

[1]TSA/XLD: TSA overlaid with Xylose Lysine Deoxycholate agar; [2]Average ± SD: average ± standard deviation; [3]Data reported at 2.5-2.7 log CFU/g are typical *Salmonella* colonies; atypical colonies at ~1.0 log CFU/g were also recovered; [4]Data reported at 2.6-3.0 log CFU/g are typical for *Salmonella* colonies; atypical colonies at 1.0-6.1 log CFU/g were also recovered; [5]Value of >3.0 was set to 3.0 log MPN/g for calculation purposes.

Table A3. Population levels of *Salmonella* recovered from artificially-inoculated garlic product samples at 2.0 log CFU/g inoculum level using three different methods between three participating laboratories. Microbiological counts are reported as log CFU/g or log MPN/g. The limit of detection is 0.0 log CFU/g by the plating method -0.5 log MPN/g by the MPN method.

Analysis	Replicate	Result log CFU/g or log MPN/g		
		Lab A	Lab B	Lab C
3-Tube MPN	A	2.0[3]	2.2	2.2
	B	2.6[3]	2.4	3.0
	C	2.2[3]	2.7	2.7
	D	2.0[3]	2.7	2.7
	E	2.4[3]	2.5	2.4
	Average ± SD[2]	2.2 ± 0.3	2.5 ± 0.2	2.6 ± 0.3
BIO-RAD Rapid *Salmonella* Agar (24 hour read)	A	1.8[4]	1.1	2.1
	B	1.9[4]	2.0	1.9
	C	1.9[4]	2.0	1.8
	D	1.8[4]	1.4	1.9
	E	1.9[4]	2.1	2.0
	Average ± SD[2]	1.9 ± 0.1	1.7 ± 0.4	1.9 ± 0.1
BIO-RAD Rapid *Salmonella* Agar (48 hour read)	A	1.8[4]	1.8	2.1
	B	1.9[4]	2.0	1.9
	C	1.9[4]	2.1	1.8
	D	1.8[4]	1.5	1.9
	E	1.9[4]	2.1	2.0
	Average ± SD[2]	1.9 ± 0.1	1.9 ± 0.2	1.9 ± 0.1
TSA/XLD[1] (24 hour read)	A	2.2[5]	1.9	2.2
	B	2.2[5]	2.2	2.4
	C	2.1[5]	2.0	2.4
	D	1.7[5]	2.2	2.5
	E	2.3[5]	2.0	2.0
	Average ± SD[2]	2.1 ± 0.2	2.1 ± 0.1	2.3 ± 0.2
TSA/XLD[1] (48 hour read)	A	2.3[5]	1.9	2.4
	B	2.3[5]	2.3	2.5
	C	2.2[5]	2.4	2.5
	D	2.1[5]	2.6	2.6
	E	2.3[5]	2.2	2.1
	Average ± SD[2]	2.2 ± 0.1	2.3 ± 0.3	2.4 ± 0.2

[1]TSA/XLD: TSA overlaid with Xylose Lysine Deoxycholate agar; [2]Average ± SD: average ± standard deviation; [3] Atypical colonies were also recovered; [4]Data reported at 1.8-1.9 log CFU/g are typical for *Salmonella* colonies; background organisms at 2.6-4.7 log CFU/g were also recovered on Bio-Rad *Salmonella* Agar; [5]Data reported at 1.7-2.3 log CFU/g are typical for *Salmonella* colonies; high levels of background organisms were also recovered on TSA/XLD ranging from 4.9->6.7 log CFU/g.

Table A4. Population levels of *Salmonella* recovered from artificially-inoculated garlic product samples at 3.0 log CFU/g inoculum level using three different methods between three participating laboratories. Microbiological counts are reported as log CFU/g or log MPN/g. The limit of detection is 0.0 log CFU/g by the plating method -0.5 log MPN/g by the MPN method.

Analysis	Replicate	Result log CFU/g or log MPN/g		
		Lab A	Lab B	Lab C
3-Tube MPN	A	3.4[3]	4.0	3.4
	B	3.2[3]	2.6	3.4
	C	3.2[3]	3.4	2.2
	D	3.2[3]	>4.0	>4.0
	E	3.6[3]	3.7	4.0
	Average ± SD[2]	3.3 ± 0.2	3.5 ± 0.6[6]	3.4 ± 0.8[6]
BIO-RAD Rapid *Salmonella* Agar (24 hour read)	A	3.0[4]	3.3	3.0
	B	2.7[4]	2.7	3.3
	C	3.0[4]	3.0	3.4
	D	3.0[4]	3.3	3.1
	E	3.1[4]	3.2	3.0
	Average ± SD[2]	2.9 ± 0.1	3.1 ± 0.2	3.2 ± 0.2
BIO-RAD Rapid *Salmonella* Agar (48 hour read)	A	3.0[4]	3.4	3.0
	B	2.8[4]	2.8	3.3
	C	3.0[4]	3.0	3.4
	D	3.0[4]	3.3	3.2
	E	3.1[4]	3.2	3.0
	Average ± SD[2]	3.0 ± 0.1	3.1 ± 0.3	3.2 ± 0.2
TSA/XLD[1] (24 hour read)	A	3.2[5]	3.8	3.0
	B	2.7[5]	3.2	3.5
	C	2.9[5]	3.4	3.4
	D	3.0[5]	3.4	3.5
	E	3.2[5]	3.2	3.3
	Average ± SD[2]	3.0 ± 0.2	3.4 ± 0.2	3.4 ± 0.2
TSA/XLD[1] (48 hour read)	A	3.3[5]	3.8	3.0
	B	2.9[5]	3.3	3.5
	C	3.2[5]	3.5	3.5
	D	3.2[5]	3.5	3.7
	E	3.4[5]	3.2	3.3
	Average ± SD[2]	3.2 ± 0.2	3.5 ± 0.2	3.4 ± 0.2

[1]TSA/XLD: TSA overlaid with Xylose Lysine Deoxycholate agar; [2]Average ± SD: average ± standard deviation; [3] Atypical colonies were also recovered; [4]Data reported at 2.7-3.1 log CFU/g are typical for *Salmonella* colonies; background organisms at 2.9-5.7 log CFU/g were also recovered on Bio-Rad *Salmonella* Agar; [5]Data reported at 2.7-3.3 log CFU/g are typical for *Salmonella* colonies; high levels of background organisms were also recovered on TSA/XLD ranging from 6.0->7.7 log CFU/g; [6]Value of >4.0 was set to 4.0 log MPN/g for calculation purposes.

Table A5. Microbiological results for the uninoculated onion product samples using three different methods between three participating laboratories. Microbiological counts are reported as log CFU/g or log MPN/g. The limit of detection is 0.0 log CFU/g by the plating method -0.5 log MPN/g by the MPN method.

Analysis	Replicate	Result (log CFU/g or log MPN/g)		
		Lab A	Lab B	Lab C
Total aerobic organisms	A	--[3]	4.7	3.6
	B	4.0	3.6	2.9
	C	4.3	3.5	4.5
	Average ± SD[2]	4.1	4.0 ± 0.7	3.7 ± 0.8
3-Tube MPN	A	<-0.5	<-0.5	<-0.5
	B	<-0.5	<-0.5	<-0.5
	C	<-0.5	<-0.5	<-0.5
BIO-RAD Rapid *Salmonella* (24 hour read)	A	<0.0	<0.0	<0.0
	B	<0.0	<0.0	<0.0
	C	<0.0	<0.0	<0.0
BIO-RAD Rapid *Salmonella* (48 hour read)	A	<0.0	<0.0	<0.0
	B	<0.0[4]	<0.0	<0.0
	C	<0.0	<0.0	<0.0
TSA/XLD[1] (24 hour read)	A	<1.0[4]	<0.0	<0.0
	B	<1.0[4]	<0.0	<0.0
	C	<1.0[4]	<0.0	<0.0
	Average ± SD[2]	<1.0	<0.0	<0.0
TSA/XLD[1] (48 hour read)	A	<1.0[4]	<0.0	<0.0
	B	<1.0[4]	<0.0	<0.0
	C	<1.0[4]	<0.0	<0.0
	Average ± SD[2]	<1.0	<0.0	<0.0

[1]TSA/XLD: TSA overlaid with Xylose Lysine Deoxycholate agar; [2]Average ± SD: average ± standard deviation; [3]Lab accident; [4]limit of detection for *Salmonella* was set to <1.0 log CFU/g; background organisms were recovered.

Table A6. Microbiological results for the uninoculated garlic product samples using three different methods between three participating laboratories. Microbiological counts are reported as log CFU/ml or log MPN/g. The limit of detection is 0.0 log CFU/g by the plating method -0.5 log MPN/g by the MPN method.

Analysis	Replicate	Result (log CFU/g or log MPN/g)		
		Lab A	Lab B	Lab C
Total aerobic organisms	A	>7.7[3]	>6.4	5.8
	B	>7.7[3]	>6.4	6.7
	C	>7.7[3]	>6.4	6.8
	Average ± SD[2]	>7.7	>6.4	6.4 ± 0.5
3-Tube MPN	A	<-0.5	<-0.5	<-0.5
	B	<-0.5	<-0.5	<-0.5
	C	<-0.5	<-0.5	<-0.5
	Average ± SD[2]	<-0.5	<-0.5	<-0.5
BIO-RAD Rapid *Salmonella* (24 hour read)	A	<3.0[4]	<0.0	<0.0
	B	<3.0[4]	<0.0	<0.0
	C	<3.0[4]	<0.0	<0.0
	Average ± SD[2]	<3.0	<0.0	<0.0
BIO-RAD Rapid *Salmonella* (48 hour read)	A	<3.0[4]	<0.0	<0.0
	B	<3.0[4]	<0.0	<0.0
	C	<3.0[4]	<0.0	<0.0
	Average ± SD[2]	<3.0	<0.0	<0.0
TSA/XLD[1] (24 hour read)	A	<4.0[4]	<0.0	<0.0
	B	<4.0[4]	<0.0	<0.0
	C	<4.0[4]	<0.0	<0.0
	Average ± SD[2]	<4.0	<0.0	<0.0
TSA/XLD[1] (48 hour read)	A	<4.0[4]	<0.0	<0.0
	B	<4.0[4]	<0.0	<0.0
	C	<4.0[4]	<0.0	<0.0
	Average ± SD[2]	<4.0	<0.0	<0.0

[1]TSA/XLD: Tryptic Soy Agar overlaid with Xylose Lysine Deoxycholate agar; [2]Average ± SD: average ± standard deviation, [3]Estimated count, counts were outside countable range on highest dilution plated; [4]limit of detection for *Salmonella* was set to either <3.0 or <4.0 log CFU/g due to high background counts.

REFERENCES

1. U.S. Food and Drug Administration (FDA). 2020. Outbreak Investigation of Salmonella Newport: Red Onions
2. U.S. Food and Drug Administration (FDA). 2021. Outbreak Investigation of Salmonella Oranienburg: Whole, Fresh Onions