Single-Shift Quantification of *Salmonella* in Chicken Carcass Rinses and Ground Poultry Meat (Chicken and Turkey) Using the Neogen® Molecular Detection Assay 2Q – Quantitative *Salmonella*

The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) is considering a regulatory framework for a strategy to control *Salmonella* in poultry products and more effectively reduce foodborne *Salmonella* infections linked to these products. One of the cross-cutting issues is to have rapid laboratory methods to determine as precisely as possible if a product is contaminated with *Salmonella* at a defined level and/or with specific serotypes of *Salmonella* as both aspects impact human illness (1).

The Neogen® Molecular Detection Assay 2 – *Salmonella* is a rapid DNA amplification method for detection of *Salmonella* in foods. This method utilizes loop-mediated isothermal amplification (LAMP) and bioluminescence to detect *Salmonella* target DNA with high specificity and sensitivity. A testing scheme/algorithm using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* (MDA2QSAL96) was developed to rapidly quantify various levels of *Salmonella* in carcass rinses and non-ready to eat chicken.

1. Performance in rinses collected in nBPW

General Procedure

Chicken carcasses artificially inoculated with various levels of *Salmonella* were rinsed with 400 mL of Neogen® neutralizing buffered peptone water (nBPW) (2). A portion of the rinse was utilized to perform Most Probable Number (MPN) according to FSIS Microbial Laboratory Guidelines (MLG) 2.05 (3). A second portion was tested using the Neogen Molecular Detection Assay 2- Quantitative *Salmonella* (MDA2QSAL96). A detailed testing scheme for this matrix can be found in the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* instructions for use.

Results

Quantification of *Salmonella* was assessed targeting three levels: 1 CFU/mL of rinse (low), 10 CFU/mL of rinse (medium) and 100 CFU/mL of rinse (high) (Table 1 and Figure 1). Method variance comparison was determined using Bonnett's test for comparison between variances and Levene's test to test for equality of variances. No statistical difference in the variance of the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* for quantification was found when compared to the MPN method at the low, medium, and high levels (Table 2).

Table 1. Quantification of *Salmonella* from nBPW rinses using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* compared to MPN.

		USDA FSIS MLG MPN Method[1,3]					Neogen Molecular Detection Assay 2Q- Quantitative *Salmonella* (MDA2QSAL96)						
Level	replicate	MPN /mL	Log 10 MPN/ mL	Log 10 Mean MPN/mL	SD	RSD (%)	MDS QSAL	Log 10 QSAL/mL	Log 10 Mean QSAL/ mL	SD	RSD (%)	Mean Dif.	95%CI
Low	1	1.9	0.26	0.220	0.09	42	2.6	0.41	0.270	0.13	50	0.05	(-.25, 0.15)
	2	1.4	0.16				2.2	0.34					
	3	1.9	0.29				1.2	0.07					
	4	1.9	0.29				2.1	0.32					
	5	1.2	0.09				1.6	0.20					
Medium	1	6.3	0.80	0.914	0.13	14	24.0	1.38	1.249	0.17	13	0.33	(-0.68, 0.02)
	2	11.3	1.05				14.3	1.15					
	3	5.8	0.76				27.5	1.44					
	4	9.5	0.98				10.7	1.03					
	5	9.5	0.98				17.5	1.24					
High	1	150	2.18	2.053	0.42	21	84.3	1.93	1.752	0.20	11	-0.30	(-0.26, 0.86)
	2	93	1.97				89.0	1.95					
	3	240	2.38				60.4	1.78					
	4	240	2.38				31.3	1.50					
	5	23	1.36				40.5	1.61					

MPN: Most Probable Number

QSAL: Quantification of *Salmonella* using Neogen Molecular Detection Assay 2Q – Quantitative *Salmonella*.

SD: Standard Deviation; RSD: Residual Standard Deviation; Mean Dif.: log mean difference between the two methods; CI: Confidence Interval.

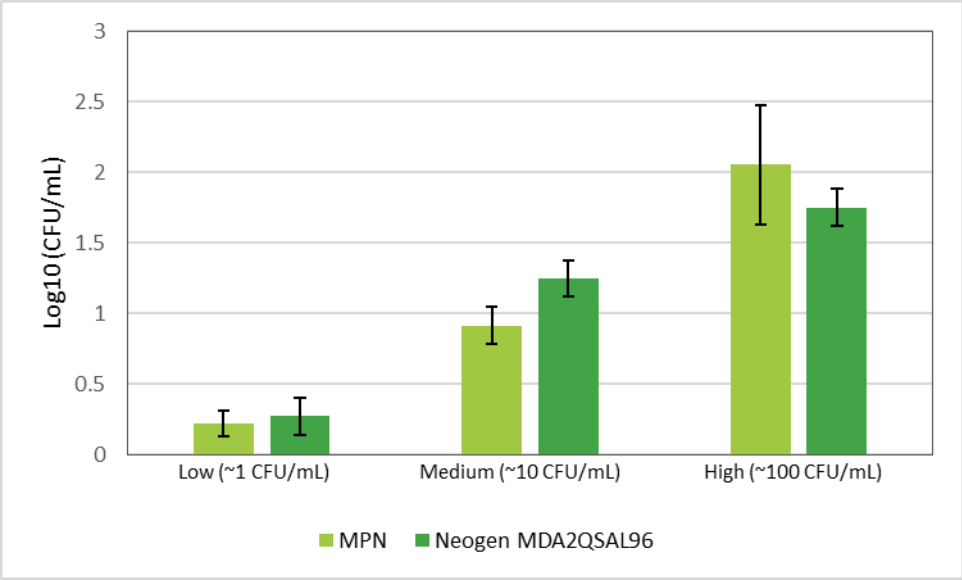

Figure 1. Quantification of *Salmonella* from nBPW rinses using the Neogen Molecular Detection Assay 2Q- Quantitative *Salmonella* compared to MPN.

Table 2. Test of variances for nBPW rinses: MPN compared to Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella*.

Variance Test	Low Level (~1CFU/mL)	Medium Level (~10CFU/mL)	High Level ~(100CFU/mL)
Bonnett's Test p-value	0.399	0.173	0.789
Levene's Test p-value	0.146	0.333	0.847

Comparison in performance between two operators over two consecutive days in the quantification of *Salmonella* was also assessed (Table 3). No statistically significant difference ($p>0.05$) was determined in the concentration of *Salmonella* using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* for quantification between operators.

Table 3. Comparison of the quantification of *Salmonella* from nBPW rinses using Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* when performed by two different operators and different testing days.

Level	Mean log 10 (CFU/mL) Operator 1 N=5	Mean log 10 (CFU/mL) Operator 2 N=5	Mean log difference (Absolute value)	p-value
Day 1				
High	1.75	1.7	0.05	0.64
Medium	1.25	1.08	0.13	0.08
Low	0.27	-0.09	0.36	0.07
Day 2				
High	1.98	2.06	0.62	0.16
Medium	1.40	1.11	0.29	0.12
Low	0.44	0.53	0.09	0.63

Construction of a binary fitted line plot demonstrated that the limit of quantification of the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* is estimated to be at 0.3 CFU/mL for nBPW rinse using a binominal regression at 95% confidence.

2. Performance in ground chicken meat

General Procedure

Test portions of ground chicken meat were homogenized with 400 mL of Neogen® BPW ISO. A portion of the generated mixture was utilized to perform the most probable number (MPN) according to the FSIS Microbiology Laboratory Guidebook (MLG) (3,4). A second portion was utilized for using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* for quantification. A detailed testing scheme can be found in the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* product instructions.

Results

Quantification of *Salmonella* was assessed targeting three levels: 1 CFU/g (low), 10 CFU/g (medium) and 100 CFU/g (high) (Table 4 and Figure 2). Method variance comparison was determined using Bonnett's test for comparison between variances and Levene's test to test for equality of variances. No statistical difference in the variance of the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* was found when compared to the MPN method at the low, medium, and high levels (Table 5).

Table 4. Quantification of *Salmonella* from ground chicken meat using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* compared to MPN.

		USDA FSIS MLG MPN Method[1,3]					Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella*(MDA2QSAL96)						
Level	replicate	MPN/g	Log 10 MPN/g	Log 10 Mean MPN/g	SD	RSD (%)	MDS QSAL	Log 10 QSAL/g	Log 10 Mean QSAL/g	SD	RSD (%)	Mean Dif.	95%CI
Low	1	1	-0.02	0.180	0.35	194	3	0.48	0.464	0.12	25	0.28	(-0.81, 0.24)
	2	1	-0.07				2	0.39					
	3	2	0.32				4	0.57					
	4	1	-0.07				4	0.57					
	5	5	0.73				2	0.31					
Medium	1	21	1.32	1.453	0.41	28	85	1.93	1.764	0.20	12	0.29	(-0.76, 0.17)
	2	100	2.00				79	1.90					
	3	17	1.23				26	1.42					
	4	10	0.98				54	1.73					
	5	54	1.73				56	1,75					
High	1	33	1.52	1.798	0.31	17	150	2.17	2.010	0.12	6	0.21	(-0.68, 0.26)
	2	210	2.32				86	1.93					
	3	52	1.72				78	1.89					
	4	52	1.72				132	2.09					
	5	52	1.72				92	1.97					

MPN: Most Probable Number

QSAL: Quantification of *Salmonella* using Neogen Molecular Detection Assay 2Q – Quantitative *Salmonella*

SD: Standard Deviation; RSD: Residual Standard Deviation; Mean Dif.: log mean difference between the two methods; CI: Confidence Interval.

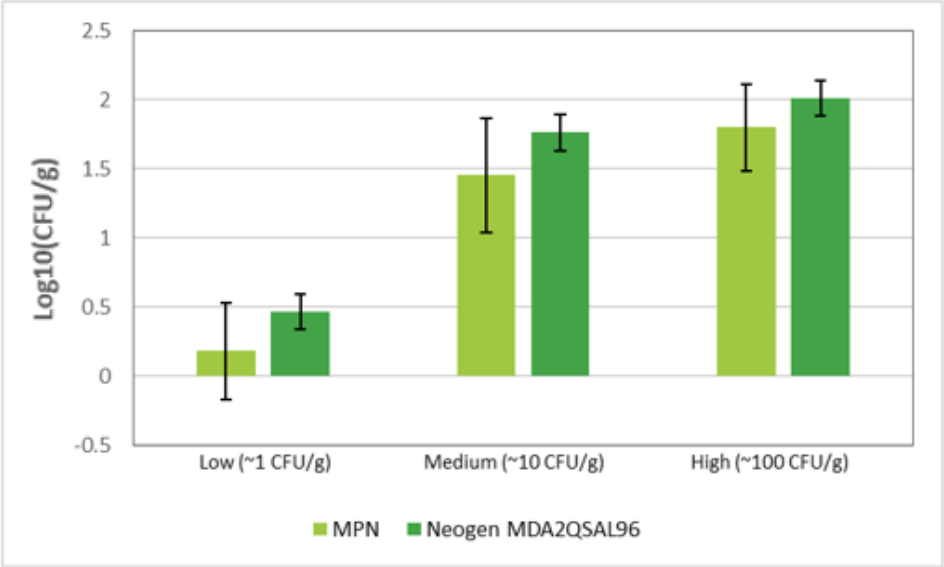

Figure 2. Quantification of *Salmonella* from ground chicken meat using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* compared to MPN.

Table 5. Test of variances for ground chicken meat: MPN and MPN prediction using Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella*.

Variance Test	Low Level (~1CFU/g)	Medium Level (~10CFU/g)	High Level ~(100CFU/g)
Bonnett's Test p-value	0.376	0.40	0.583
Levene's Test p-value	0.594	0.275	0.594

Comparison in performance between two operators over two consecutive days in the quantification of *Salmonella* was also assessed (Table 6). No statistically significant difference ($p>0.05$) was determined in the concentration of *Salmonella* using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* between operators.

Table 6. Comparison of the quantification of *Salmonella* from ground chicken meat using Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* when performed by two different operators and different testing days.

Level	Mean log 10 (CFU/g) Operator 1 N=5	Mean log 10 (CFU/g) Operator 2 N=5	Mean log difference (Absolute value)	p-value
Day 1				
High	0.33	0.46	-0.13	0.09
Medium	1.48	1.75	-0.27	0.09
Low	1.75	2.01	-0.25	0.26
Day 2				
High	0.58	0.53	0.05	0.675
Medium	1.27	1.19	0.07	0.347
Low	1.85	1.77	0.08	0.456

Construction of a binary fitted line plot demonstrated that the limit of quantification of the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* is estimated to be at 0.66 CFU/g for ground chicken meat using a binominal regression at 95% confidence.

3. Performance in rinses collected in BPW

General Procedure

Chicken carcasses artificially inoculated with various levels of *Salmonella* were rinsed with 400 mL of buffered peptone water (BPW). A portion of the rinse was utilized to perform Most Probable Number (MPN) according to FSIS Microbial Laboratory Guidelines (MLG) 2.05 (3). A second portion was utilized to be tested using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* (MDA2QSAL96). A detailed testing scheme for this matrix can be found in the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* instructions for use.

Results

Quantification of *Salmonella* was assessed targeting three levels: 1 CFU/mL (low), 10 CFU/mL (medium) and 100 CFU/mL (high) (Table 7 and Figure 3). Method variance comparison was determined using Bonnett's test. No statistical difference in the variance of the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* was found when compared to the MPN method at the medium, and high levels (Table 8). Significant difference in the variance was found for the low level which is associated to higher variability in the log CFU/mL results

for both methods. Paired t-test of the study comparison was not significant difference between the alternative and reference method (p=0.277).

Table 7. Quantification of *Salmonella* from BPW rinses using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* compared to MPN.

		USDA FSIS MLG MPN Method[1,3]					Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella*(MDA2QSAL96)						
Level	replicate	MPN/ mL	Log 10 MPN/g	Log 10 Mean MPN/g	SD	RSD (%)	MDS QSAL	Log 10 QSAL/g	Log 10 Mean QSAL/g	SD	RSD (%)	Mean Dif.	95%CI
Low	1	1.10	0.041	-0.396	0.256	64.8	1.04	0.016	0.090	0.092	102	0.485	0.240, 0.731
	2	0.34	-0.469				1.55	0.191					
	3	0.34	-0.469				1.02	0.010					
	4	0.36	-0.444				1.54	0.189					
	5	0.23	-0.638				1.10	0.043					
Medium	1	3.60	0.556	0.640	0.117	18.2	5.06	0.704	0.721	0.143	19.8	0.081	-0.115, 0.276
	2	5.50	0.740				4.99	0.698					
	3	6.20	0.792				7.25	0.860					
	4	3.60	0.556				3.18	0.503					
	5	3.60	0.556				6.90	0.839					
High	1	62.00	1.792	1.684	0.323	19.1	21.01	1.322	1.464	0.120	8.2	0.221	-0.616, 0.175
	2	62.00	1.792				24.60	1.391					
	3	23.00	1.362				26.83	1.429					
	4	23.00	1.362				39.75	1.599					
	5	130	2.114				37.75	1.577					

MPN: Most Probable Number

QSALl: Quantification of *Salmonella* using Neogen Molecular Detection Assay 2Q – Quantitative *Salmonella*

SD: Standard Deviation; RSD: Residual Standard Deviation; Mean Dif.: log mean difference between the two methods; CI: Confidence Interval.

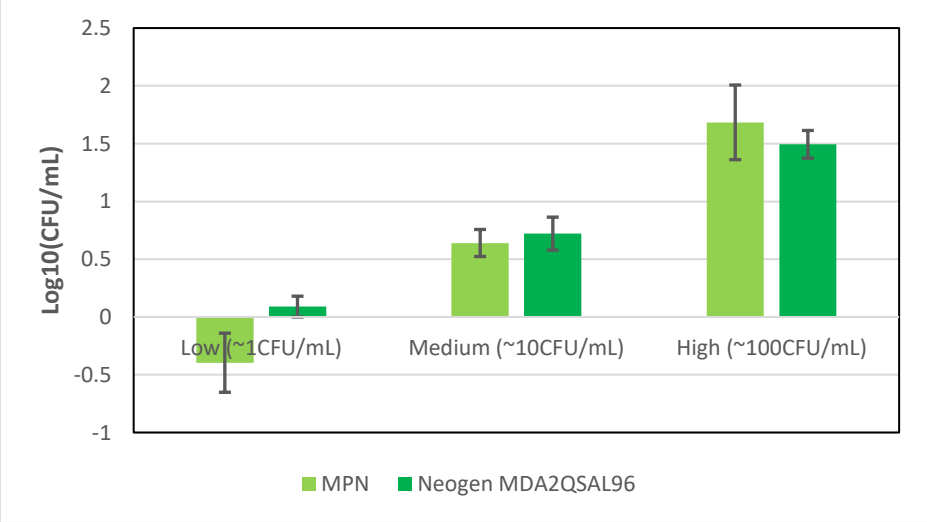

Figure 3. Quantification of *Salmonella* from BPW rinses using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* compared to MPN.

Table 8. Test of variances for BPW rinses: MPN and MPN prediction using Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella*.

Variance Test	Low Level (~1CFU/mL)	Medium Level (~10CFU/mL)	High Level ~(100CFU/mL)
Bonnett's Test p-value	0.067	0.760	0.779
Levene's Test p-value	0.045	0.731	0.604

Comparison in performance between two operators in the quantification of *Salmonella* was also assessed (Table 9). No statistically significant difference ($p>0.05$) was determined in the concentration of *Salmonella* using the Neogen Molecular Detection Assay 2- Quantitative *Salmonella* between operators.

Table 9. Comparison of the quantification of *Salmonella* from BPW rinses using Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* when performed by two different operators and different testing days.

Level	Mean log 10 (CFU/mL) Operator 1 N=5	Mean log 10 (CFU/mL) Operator 2 N=5	Mean log difference (Absolute value)	p-value Analysis of Means (operator effect)
Day 1				
High	-0.115	-0.151	0.036	0.937
Medium	0.678	0.893	0.251	
Low	1.120	1.014	0.105	

**Due to available materials, the comparison operator to operator was only performed for one day of testing.*

Construction of a binary fitted line plot demonstrated that the limit of quantification of the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* is estimated to be at 0.3 CFU/mL for BPW rinse using a binominal regression at 95% confidence.

4. Performance in ground turkey meat

General Procedure

Ground turkey meat (325 g test portion) was artificially inoculated with various levels of *Salmonella* and homogenized with 400 mL of buffered peptone water (BPW). A portion of the homogenate was utilized to perform Most Probable Number (MPN) according to FSIS Microbial Laboratory Guidelines (MLG) 2.05 (3). A second portion was utilized to be tested using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* (MDA2QSAL96). A detailed testing scheme for this matrix can be found in the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* instructions for use.

Results

Quantification of *Salmonella* was assessed targeting three levels: 1 CFU/g (low), 10 CFU/g (medium) and 100 CFU/g (high) (Table 10 and Figure 4). Method variance comparison was determined using Bonnett's test. No statistical difference in the variance of the Neogen Molecular Detection Assay 2- Quantitative *Salmonella* was found when compared to the MPN method at the low, medium, and high levels (Table 11).

Table 10. Quantification of *Salmonella* from ground turkey meat samples using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* compared to MPN.

		USDA FSIS MLG MPN Method[1,3]					Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella*(MDA2QSAL96)						
Level	replicate	MPN/g	Log 10 MPN/g	Log 10 Mean MPN/g	SD	RSD (%)	MDS QSAL	Log 10 QSAL/g	Log 10 Mean QSAL/g	SD	RSD (%)	Mean Dif.	95%CI
Low	1	2.9	0.462	0.149	0.276	184	3.8	0.582	0.488	0.166	33.8	0.339	-0.691, 0.012
	2	2.6	0.415				2.8	0.444					
	3	1.2	0.079				3.1	0.498					
	4	0.8	-0.092				4.8	0.680					
	5	0.8	-0.119				1.7	0.239					
Medium	1	8.1	0.908	0.827	0.236	28	9.3	0.967	0.814	0.191	23.4	0.013	-0.308, 0.333
	2	8.1	0.908				8.5	0.931					
	3	12.0	1.079				5.7	0.756					
	4	2.8	0.447				8.2	0.913					
	5	6.2	0.792				3.2	0.505					
High	1	81.0	1.908	1.609	0.374	21.5	18.6	1.271	1.345	0.208	15.5	0.264	-0.707, 0.178
	2	52.0	1.716				11.9	1.075					
	3	25.0	1.398				20.1	1.304					
	4	13.0	1.114				27.4	1.438					
	5	81.0	1.908				43.1	1.635					

MPN: Most Probable Number

QSAL: Quantification of *Salmonella* using Neogen Molecular Detection Assay 2Q – Quantitative *Salmonella*

SD: Standard Deviation; RSD: Residual Standard Deviation; Mean Dif.: log mean difference between the two methods; CI: Confidence Interval.

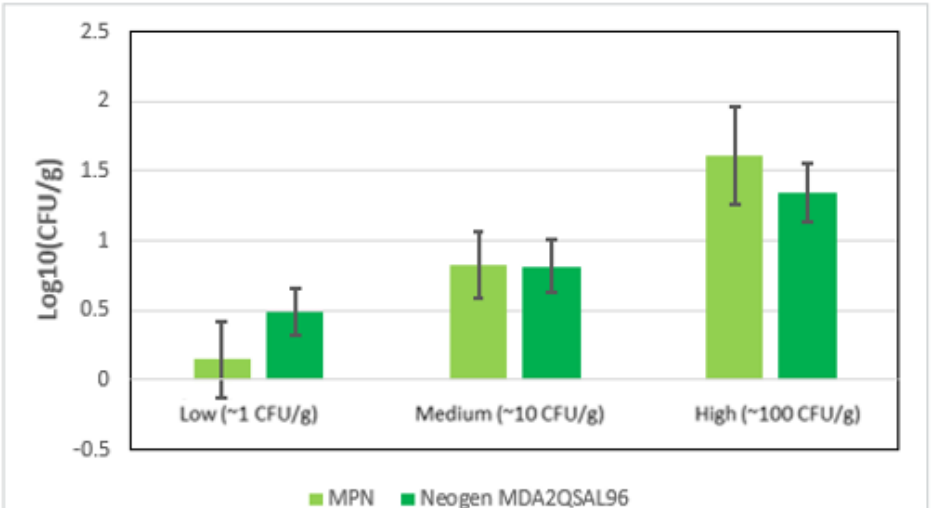

Figure 4. Quantification of *Salmonella* from ground turkey meat using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* compared to MPN.

Table 11. Test of variances for ground turkey meat samples: MPN and MPN prediction using Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella*.

Variance Test	Low Level (~1CFU/g)	Medium Level (~10CFU/g)	High Level ~(100CFU/g)
Bonnett's Test p-value	0.955	0.952	0.112
Levene's Test p-value	0.316	0.707	0.129

Comparison in performance between two operators in the quantification of *Salmonella* was also assessed (Table 12). No statistically significant difference ($p>0.05$) was determined in the concentration of *Salmonella* using the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* between operators.

Table 12. Comparison of the quantification of *Salmonella* from ground turkey meat samples using Neogen Molecular Detection Assay 2- Quantitative *Salmonella* when performed by two different operators and different testing days.

Level	Mean log 10 (CFU/g) Operator 1 N=5	Mean log 10 (CFU/g) Operator 2 N=5	Mean log difference (Absolute value)	p-value Analysis of Means (operator effect)
Day 1				
Low	-0.065	-0.450	0.384	0.443
Medium	0.863	0.835	0.027	
High	1.422	1.492	0.070	
Day 2				
Low	0.423	0.217	0.205	0.782
Medium	0.908	1.032	0.123	
High	1.405	1.571	0.166	

Construction of a binary fitted line plot demonstrated that the limit of quantification of the Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* is estimated to be at 0.68 CFU/g for ground turkey meat using a binominal regression at 95% confidence.

Additional Notes:

For all matrices tested in this study, additional analysis were performed to demonstrate that quantification was not significantly different between commercial lots of Neogen Molecular Detection Assay 2Q - Quantitative *Salmonella* test kits, among multiple days of testing, and between different Neogen® Molecular Detection Instruments tested.

Conclusions

The Neogen Molecular Detection Assay 2Q- Quantification *Salmonella*, predicted the concentration of *Salmonella* from carcass rinses collected in nBPW and BPW as well for ground poultry meat samples (chicken and turkey) with a log mean difference of less than 0.5 and no difference in method variance compared to MPN variance.

- This method showed repeatability between operators and different days of testing.
- This method is robust to various assay conditions including lot to lot of reagent kits.

Thus, the Neogen Molecular Detection Assay 2Q – Quantification *Salmonella* enables a faster and less laborious method than traditional Most Probable Number to determine the concentration of *Salmonella* in chicken rinses and ground poultry meat (chicken and turkey). This can allow poultry producers to assess the effect of interventions to control *Salmonella and/*or implement early intervention to minimize the risk of *Salmonella* in product.

Note: The data presented in this performance summary was collected internally.

References:

(1) USDA FSIS. (2024) . Proposed Regulatory Framework to Reduce *Salmonella* Illnesses Attributable to Poultry. Proposed Regulatory Framework to Reduce Salmonella Illnesses Attributable to Poultry (usda.gov) Accessed 6.22.24.

(2) USDA FSIS (2021). Sampling Instructions: *Salmonella* and *Campylobacter* verification program for raw poultry products. FSIS Directive 10,250.1. Rev 1. FSIS Directive 10,250.1 Rev 1 Sampling Instructions Salmonella and Campylobacter Verification Program for Raw Poultry Products (usda.gov). Accessed 6.22.24.

(3) USDA FSIS. (2023). Isolation and Identification of *Salmonella* from meat, poultry, pasteurized egg, siluriformes (Fish) products and carcasses and environmental sponges. MLG 4.14 Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, Siluriforms (Fish) Products and Carcass and Environmental Sponges (usda.gov). Accessed 6.23.24.

(4) USDA FSIS. (2014). Most Probable Number Procedure and Tables. Microbiology Laboratory Guidebook. MLG Appendix 2.05. Most Probable Number Procedure and Tables. (usda.gov) . Accessed 6.22.24

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