Quantification and Limits Manual

Instructions for Specific Matrices and Pathogen Targets

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Required Materials

Product Number	Product	Storage Conditions
KIT2039	BAX® System Real-Time PCR Assay for *E. coli* O157:H7 EXACT (96 tests)	2 – 8 °C
KIT2005	BAX System Real-Time PCR Assay for *L. monocytogenes* (96 tests)	2 – 8 °C
KIT2006	BAX System Real-Time PCR Assay for *Salmonella* (96 tests)	2 – 8 °C
KIT2010	BAX System Real-Time PCR Assay for *Vibrio cholerae/parahaemolyticus/vulnificus* (96 tests)	2 – 8 °C
KIT2018	BAX System Real-Time PCR Assay for *Campylobacter jejuni/coli/lari* (96 tests)	2 – 8 °C
KIT2019	BAX System Real-Time PCR Assay for Genus *Listeria* (96 tests)	2 – 8 °C
MED2010	Buffered Peptone Water (2.5 kg)	Room Temperature: 2 – 30 °C
MED2003 MED2016 MED2029	BAX System MP Media (2.5 kg, 10 kg, STAT packs)	Room Temperature: 10 – 25 °C
MED2032	BAX System Quant™ Solution (25 mL)	2 – 8 °C
ASY2018 ASY2020	BAX System Q7 Start-Up Package (Equipment and supplies for 192 initial tests; 120/220V)	Specifications available at www.hygiena.com
STC SUBS- PREM	SureTrend® Quant (Part of SureTrend Cloud Premium (SaaS))	Specifications available here
MT-S100	MicroTally® Swabs (or similar)	Purchase from vendor directly

References throughout: *mLOQ = the minimum level of quantification and requires calculations
**Limits LOD = the limit of detection for a positive/negative result

Note: MicroTally® is a registered trademark of Fremonta

Poultry Testing

Quantification of *Salmonella*

Testing was performed using the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
Boot Swab	Internal validation	1 boot swab	10	1 CFU/mL	1 - 10,000	Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Ceca	Internal validation	1 cecal tonsil	10	10 CFU/mL	10 - 10,000	Add 1 cecal tonsil to pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Cloacal Swabs	Internal validation	10 cloacal swabs	10	10 CFU/mL	10 - 10,000	Add a composite of 10 cloacal swabs to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
Crop	Internal validation	1 crop	6	10 CFU/g	10 - 10,000	Add 1 poultry crop to 400 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Dust Swab	Internal validation	1 dust swab	10	10 CFU/mL	10 - 10,000	Add 1 dust swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Feed	Internal validation	25 g	8	10 CFU/g	10 - 10,000	Add 25 g of poultry (turkey) feed to 225 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant™ Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Feet Swabs	Internal validation	5 turkey feet swabs	10	10 CFU/mL	10 - 10,000	Add a composite of 5 turkey feet swabs to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
Liver	Internal validation	1 liver	6	1 CFU/mL	1 - 10,000	Add 1 poultry liver to 200 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Lungs	Internal validation	1 lung	6	10 CFU/g	10 - 10,000	Add 1 poultry lung to 300 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Poult Pads	Internal validation	25 g	8	1 CFU/g	1 - 1,000	Add 25 g of poult pads (cardboard or straw) to 750 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Spleen	Internal validation	1 spleen	6	1 CFU/mL	1 - 10,000	Add 1 poultry spleen to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
Carcass Swabs	Internal validation	1 carcass swab	6	1 CFU/mL	1 - 10,000	Add 1 carcass swab to 50 mL of pre-warmed (42 °C) BPW media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Rinsate	AOAC *PTM*[SM] 081201	30 mL rinse	6	1 CFU/mL	1 - 10,000	Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Rinsate (Low Level)	Internal validation	30 mL rinse	10	0.5 CFU/ 30 mL sample	0.5 - 31	Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 10 h for 0.5 - 31 CFU/30 mL. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Ground Chicken 1:4	AOAC *PTM*[SM] 081201	325 g	8	1 CFU/g	1 - 1,000	Add 325 g of comminuted chicken to 325 mL (1:1) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
Ground Chicken 1:6	AOAC *PTM*SM 081201	325 g	8	1 CFU/g	1 - 1,000	Add 325 g of comminuted chicken to 975 mL (1:4) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Ground Turkey 1:1	AOAC *PTM*SM 081201	325 g	8	1 CFU/g	1 - 1,000	Add 325 g of comminuted turkey to 325 mL (1:1) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Ground Turkey 1:4	AOAC *PTM*SM 081201	325 g	8	1 CFU/g	1 - 1,000	Add 325 g of comminuted turkey to 975 mL (1:4) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
Ground Turkey 1:6	AOAC *PTM*[SM] 081201	325 g	8	1 CFU/g	1 - 1,000	Add 325 g of comminuted turkey to 1,625 mL (1:6) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Breaded Stuffed Raw Chicken	Internal validation	325 g	6	1 CFU/mL	1 - 10,000	Add 375 g of breaded and stuffed raw chicken product to 375 mL of pre-warmed (42 °C) BPW media. Hand massage for 30 seconds for homogenization. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX® MP media containing 0.5 mL/L of BAX® Quant Solution. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Limits Testing for *Salmonella*

Testing was performed using the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Boot Swab	Internal validation	1 boot swab	10	LOD1	Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Ceca	Internal validation	1 cecal tonsil	10	LOD10	Add 1 cecal tonsil to pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Cloacal Swabs	Internal validation	10 cloacal swabs	10	LOD10	Add a composite of 10 cloacal swabs to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Crop	Internal validation	1 crop	6	LOD10	Add 1 poultry crop to 400 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Dust Swab	Internal validation	1 dust swab	10	LOD10	Add 1 dust swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Feed	Internal validation	25 g	8	LOD10	Add 25 g of poultry (turkey) feed to 225 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant™ Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Feet Swabs	Internal validation	5 turkey feet swabs	10	LOD10	Add a composite of 5 turkey feet swabs to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Liver	Internal validation	1 liver	6	LOD1	Add 1 poultry liver to 200 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Lungs	Internal validation	1 lung	6	LOD10	Add 1 poultry lung to 300 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Poult Pads	Internal validation	25 g	8	LOD1	Add 25 g of poult pads (cardboard or straw) to 750 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Spleen	Internal validation	1 spleen	6	LOD1	Add 1 poultry spleen to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Carcass Swabs	Internal validation	1 carcass swab	6	LOD1	Add 1 carcass swab to 50 mL of pre-warmed (42 °C) BPW media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following Incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Rinsate	Internal validation	30 mL rinse	4	LOD10	Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 4 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Rinsate (Low level)	Internal validation	30 mL rinse	10	LOD 0.5/30 mL	Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 4 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Chicken 1:4	AOAC *PTM*℠ 081201	325 g	8	LOD1	Add 325 g of comminuted chicken to 325 mL (1:1) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Ground Chicken 1:6	AOAC *PTM*[SM] 081201	325 g	8	LOD1	Add 325 g of comminuted chicken to 975 mL (1:4) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Turkey 1:1	AOAC *PTM*[SM] 081201	325	6	LOD10	Add 325 g of comminuted turkey to 325 mL (1:1) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Turkey 1:1	AOAC *PTM*[SM] 081201	325	8	LOD1	Add 325 g of comminuted turkey to 325 mL (1:1) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Turkey 1:4	AOAC *PTM*[SM] 081201	325	8	LOD1	Add 325 g of comminuted turkey to 975 mL (1:4) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Ground Turkey 1:6	AOAC *PTM*[SM] 081201	325 g	8	LOD1	Add 325 g of comminuted turkey to 1,625 mL (1:6) of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Chicken Skin	Internal validation	325 g	6	LOD1	Add 325 g of comminuted chicken to 975 mL (1:4) of BPW or nBPW. Transfer 30 mL of the solution into 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Chicken Breast	Internal validation	325 g	6	LOD1	Add 325 g of comminuted chicken to 975 mL (1:4) of BPW or nBPW. Transfer 30 mL of the solution into 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Breaded Stuffed Raw Chicken	Internal Validation	325 g	6	LOD1	Add 375 g of breaded and stuffed raw chicken product to 375 mL of pre-warmed (42 °C) BPW media. Hand massage for 30 seconds for homogenization. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX® MP media containing 0.5 mL/L of BAX® Quant Solution. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Turkey Breast	Internal validation	325 g	6	LOD5	Add 325 g of turkey breasts to 975 mL (1:4) of BPW. Transfer 30 mL of the solution into 30 mL of pre-warmed (42 °C) BPW. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Turkey Thighs	Internal validation	325 g	6	LOD5	Add 325 g of turkey thighs to 975 mL (1:4) of BPW. Transfer 30 mL of the solution into 30 mL pre-warmed (42 °C) BPW. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Turkey Wings	Internal Validation	325 g	6	LOD5	Add 325 g of turkey wings to 975 mL (1:4) of BPW. Transfer 30 mL of the solution into 30 mL of pre-warmed (42 °C) BPW. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Quantification of *Campylobacter*

Testing was performed using the BAX System Real-Time PCR Assay for *Campylobacter* (KIT2018)

Campylobacter jejuni, C. coli, C. lari

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantification of *Campylobacter* in Poultry
Rinsate	Internal validation	30 mL rinse	20	10 CFU/mL	10 - 10,000	Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (45 °C) 2X Bolton's Broth + 2X supplement. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 20 h in microaerophilic conditions. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Utilize SureTrend Quant for result calculations.
Chicken Neck Skins	Internal validation	25 g	16	1 CFU/g	1-1,000	Add 25 g of chicken neck skins to 250 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 10 mL of the Primary Enrichment into a sterile container with 10 mL of pre-warmed (45 °C) 2X Bolton's Broth + 2X supplement. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 16 h under microaerophilic conditions. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Utilize SureTrend Quant for result calculations.

Limits Testing for *Campylobacter*

Testing was performed using the BAX System Real-Time PCR Assay for *Campylobacter* (KIT2018)

Campylobacter jejuni, C. coli, C. lari

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Campylobacter* in Poultry
Rinsate	Internal validation	30 mL rinse	20	LOD10	Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (45 °C) 2X Bolton's Broth with 2X supplement. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 20 h in microaerophilic conditions. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Chicken Neck Skins	Internal validation	25 g	16	LOD1	Add 25 g of chicken neck skins to 250 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 10 mL of the Primary Enrichment into a sterile container with 10 mL of pre-warmed (45 °C) 2X Bolton's Broth with 2X supplement. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 16 h under microaerophilic conditions. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Beef Testing

Quantification of *Salmonella*

Testing was performed using the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantifications of *Salmonella* in Beef
Boot Swab	Internal validation	1 boot swab	6	1 CFU/mL	1 - 10,000	Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Feces	Internal validation	10 g	8	10 CFU/g	10 - 10,000	Add 10 g of beef feces to 90 mL of pre-warmed 42 °C BAX MP with 0.5 mL/L Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Feces - High Level	Internal validation	10 g	0	100,000 CFU/g	100,000 - 100,000,000	Add 10 g of beef feces to 90 mL of pre-warmed 42 °C BAX MP with 0.5 mL/L Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 0 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantifications of *Salmonella* in Beef
Micro-Tally® Drain Swab	Internal validation	1 Micro-tally	6	1 CFU/mL	1 - 10,000	Add 1 MicroTally to 200 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Carcass Swab	Internal validation	1 swab	8	10 CFU/swab	10 - 10,000	Swab a beef carcass with a BPW pre-moistened swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Cecal Contents (Low Level)	Internal validation	10 g	8	1 CFU/g	1 - 1,000	Add 10 g of beef cecal contents to 90 mL of BAX MP with + 0.5 mL/L Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 10 mL of the Primary Enrichment into a sterile container with 10 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Cecal Contents (High Level)	Internal validation	10 g	0	100,000 CFU/g	100,000 - 100,000,000	Add 10 g of beef cecal contents to 90 mL of BAX MP with + 0.5 mL/L Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 10 mL of the Primary Enrichment into a sterile container with 10 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 0 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantifications of *Salmonella* in Beef
Cecal Swab	Internal validation	1-25 mL pre-moistened BPW swab	8	10 CFU/mL	10 - 10,000	Swab beef ceca with a 25 mL pre-moistened BPW swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Lymph Nodes	Internal validation	< 10 g lymph node	6	10 CFU/ lymph node	10 - 10,000	Weigh and process lymph nodes into small (<10 g) or medium (>10 g) size category. For small nodes, add 40 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Lymph Nodes	Internal validation	> 10 g lymph node	6	10 CFU/ lymph node	10 - 10,000	Weigh and process lymph nodes into small (<10 g) or medium (>10 g) size category. For medium nodes, add 80 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Micro-Tally (for trim)	AOAC *PTM*[SM] 081201	1 Micro-Tally swab	6	1 CFU/mL	1 - 10,000	Add 1 MicroTally (swabbed on beef trim) to 200 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Trim	AOAC *PTM*[SM] 081201	375 g	6	1 CFU/g	1 - 10,000	Add 375 g of beef trim to 1,500 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantifications of *Salmonella* in Beef
Ground Beef	AOAC *PTM*[SM] 081201	375 g	6	1 CFU/g	1 - 10,000	Add 375 g of ground beef to 1,500 mL of prewarmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Limits Testing for *Salmonella*

Testing was performed using the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Beef
Boot swab	Internal validation	1 boot swab	6	LOD1	Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Feces	Internal validation	10 g	8	LOD 10	Add 10 g of beef feces to 90 mL of pre-warmed 42 °C BAX MP + 0.5 mL/L Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Feces – High level	Internal validation	10 g	0	LOD 100,000	Add 10 g of beef feces to 90 mL of pre-warmed 42 °C BAX MP + 0.5 mL/L Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 0 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Carcass Swab	Internal validation	1 swab	8	LOD10	Swab a beef carcass with a BPW pre-moistened swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Beef
Cecal Contents (Low Level)	Internal validation	10 g	8	LOD1	Add 10 g of beef cecal contents to 90 mL of BAX MP with + 0.5 mL/L Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 10 mL of the Primary Enrichment into a sterile container with 10 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Cecal Contents (High Level)	Internal validation	10 g	0	LOD 100,000	Add 10 g of beef cecal contents to 90 mL of BAX MP with + 0.5 mL/L Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 10 mL of the Primary Enrichment into a sterile container with 10 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 0 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Cecal Swab	Internal validation	1-25 mL pre-moistened BPW swab	8	LOD10	Swab beef ceca with a 25 mL pre-moistened BPW swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Lymph Nodes	Internal validation	< 10 g lymph node	6	LOD10	Weigh and process lymph nodes into small (<10 g) or medium (>10 g) size category. For small nodes, add 40 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Beef
Lymph Nodes	Internal validation	> 10 g lymph node	6	LOD10	Weigh and process lymph nodes into small (<10 g) or medium (>10 g) size category. For medium nodes, add 80 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Micro-Tally (for trim)	Internal validation	1 Micro-Tally swab	4	LOD10	Add 1 MicroTally (swabbed on beef trim) to 200 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 4 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Micro-Tally (for trim)	Internal validation	1 Micro-Tally swab	6	LOD1	Add 1 MicroTally (swabbed on beef trim) to 200 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Trim	Internal validation	375 g	4	LOD10	Add 375 g of beef trim to 1,500 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 4 h for LOD10. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Beef	Internal validation	375 g	4	LOD10	Add 375 g of ground beef to 1,500 mL of prewarmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 4 h for LOD10. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Quantification of *E. coli* O157:H7

Testing was performed using the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact (KIT2039)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure - Quantification of E. coli O157:H7 in Beef
Carcass Swab	Internal validation	1 swab	8	10 CFU/Swab	10 - 10,000	Swab a beef carcass with a BPW pre-moistened swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *E. coli* O157:H7 Exact. Utilize SureTrend Quant for result calculations.

Limits Testing for *E. coli* O157:H7

Testing was performed using the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact (KIT2039)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD *Positive/ Negative Result	Procedure - Limits Testing for E. coli O157:H7 in Beef
Carcass Swab	Internal validation	1 swab	8	LOD10	Swab a beef carcass with a BPW pre-moistened swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *E. coli* O157:H7 Exact. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Pork Testing

Quantification of *Salmonella*

Testing was performed using the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Pork
Boot Swab	Internal validation	1 boot swab	8	1 CFU/mL	1 - 10,000	Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Feces	Internal validation	10 g	10	1 CFU/g	1 - 10,000	Add 10 g of pork feces to 90 mL of pre-warmed (42 °C) BAX MP with 0.5 mL/L of BAX Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Feces - High Level	Internal validation	10 g	0	100,000 CFU/g	100,000 - 100,000,000	Add 10 g of pork feces to 90 mL of pre-warmed (42 °C) BAX MP + 0.5 mL/L of BAX Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 30 seconds for homogenization. Test immediately - proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Pork
Carcass Swab	Internal validation	1 carcass swab	6	1 CFU/mL	1 - 1,000	Add 1 carcass swab to 50 mL of BPW as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Head Trim Rinsate	Internal validation	1 lb. head trim	7	1 CFU/mL	1 - 1,000	Add 1 lb. (453.6 g) of head trim to 400 mL of BPW as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 7 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Lymph Nodes	Internal validation	0-3 g lymph node	6	10 CFU/ lymph node	10 - 100,000	Weigh and process lymph nodes into small (0 - 3 g) or medium (3.1 - 25 g) size categories. For small nodes, add 20 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Lymph Nodes	Internal validation	3.1-25 g lymph node	6	10 CFU/ lymph node	10 - 100,000	Weigh and process lymph nodes into small (0 - 3 g) or medium (3.1 - 25 g) size categories. For medium nodes, add 80 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Pork
Ground Pork	AOAC *PTM*SM 081201	375 g	7	1 CFU/g	1 - 1,000	Add 375 g of ground pork to 1,500 mL of prewarmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 7 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Micro-Tally (for trim)	AOAC *PTM*SM 081201	1 Micro-Tally swab	6	1 CFU/mL	1 - 10,000	Add 1 MicroTally (swabbed on pork trim) to 200 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.
Trim	AOAC *PTM*SM 081201	375 g	6	1 CFU/g	1 - 10,000	Add 375 g of pork trim to 1,500 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Limits Testing for *Salmonella*

Testing was performed using the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Pork
Boot Swab	Internal validation	1 boot swab	8	LOD1	Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Feces	Internal validation	10 g	10	LOD1	Add 10 g of pork feces to 90 mL of pre-warmed (42 °C) BAX MP + 0.5 mL/L of BAX Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Feces - High Level	Internal validation	10 g	0	LOD 100,000	Add 10 g of pork feces to 90 mL of pre-warmed (42 °C) BAX MP + 0.5 mL/L of BAX Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 10 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 30 seconds for homogenization. Test immediately. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Carcass Swab	Internal validation	1 carcass swab	4	LOD10	Add 1 carcass swab to 50 mL of BPW as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 4 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Pork
Head Trim Rinsate	Internal validation	1 lb. head trim	7	LOD1	Add 1 lb. (453.6 g) of head trim to 400 mL of BPW as Primary Enrichment. Homogenize by hand for 60 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 7 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Lymph Nodes	Internal validation	0-3 g lymph node	6	LOD10	Weigh and process lymph nodes into small (0 - 3 g) or medium (3.1 - 25 g) size categories. For small nodes, add 20 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Lymph Nodes	Internal validation	3.1-25 g lymph node	6	LOD10	Weigh and process lymph nodes into small (0 - 3 g) or medium (3.1 - 25 g) size categories. For medium nodes, add 80 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Pork	Internal validation	375 g	4	LOD10	Add 375 g of ground pork to 1,500 mL of prewarmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 5 h for LOD10. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Pork
Micro-Tally (for trim)	Internal validation	1 Micro-Tally swab	4	LOD10	Add 1 MicroTally (swabbed on pork trim) to 200 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 4 h for LOD10. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Trim	Internal validation	375 g	4	LOD10	Add 375 g of pork trim to 1,500 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 4 h for LOD10. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Seafood Testing

Quantification of *Vibrio*

Testing was performed using the BAX System Real-Time PCR Assay for *Vibrio* (KIT2010)

Vibrio parahaemolyticus, V. vulnificus, V. cholerae

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Vibrio* in Seafood
Oysters	Internal validation	25 g	8	1 CFU/g	1 - 1,000	Add 1 MicroTally (swabbed on oysters) to 250 mL of pre-warmed (42 °C) Alkaline Peptone Water (APW) media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Vibrio*. Utilize SureTrend Quant for result calculations.

Limits Testing for *Vibrio*

Testing was performed using the BAX System Real-Time PCR Assay for *Vibrio* (KIT2010)

Vibrio parahaemolyticus, V. vulnificus, V. cholerae

Sample Type	Validation	Sample Size (g)	Time-point (h)	Limits LOD *Positive/ Negative Result	Procedure – Limits Testing for *Vibrio* in Seafood
Oysters	Internal validation	25 g	6	LOD10	Add 1 MicroTally (swabbed on oysters) to 250 mL of pre-warmed (42 °C) Alkaline Peptone Water (APW) media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h for LOD10. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Vibrio*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.
Oysters	Internal validation	25 g	8	LOD1	Add 1 MicroTally (swabbed on oysters) to 250 mL of pre-warmed (42 °C) Alkaline Peptone Water (APW) media as Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h for LOD10. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Vibrio*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Produce Testing

Quantification of *Listeria*

Testing was performed using the BAX System Real-Time PCR Assay for Genus *Listeria* (KIT2019) -or- the BAX System Real-Time PCR Assay for *L. mono* (KIT2005)

Listeria spp., L. monocytogenes

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Listeria* in Produce
Lettuce	Internal validation	125 g	16	1 CFU/g	1 - 1,000	Add 125 g of lettuce to 1,125 mL of prewarmed (37 °C) 24 LEB Complete media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 35 ± 1 °C for 16 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for Genus *Listeria* or *L. mono*. Utilize SureTrend Quant for result calculations.

Limits Testing for *Listeria*

Testing was performed using the BAX System Real-Time PCR Assay for Genus *Listeria* (KIT2019) -or- the BAX System Real-Time PCR Assay for *L. mono* (KIT2005)

Listeria spp., L. monocytogenes

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD *Positive/ Negative Result	Procedure – Limits Testing for *Listeria* in Produce
Lettuce	Internal validation	125 g	16	LOD1	Add 125 g of lettuce to 1,125 mL of prewarmed (37 °C) 24 LEB Complete media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 35 ± 1 °C for 16 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for Genus *Listeria* or *L. mono*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Environmental Monitoring Testing

Quantification of *Listeria*

For *Listeria* - Testing was performed using the BAX System Real-Time PCR Assay for Genus *Listeria* (KIT2019) -or- the BAX® System Real-Time PCR Assay for *L. mono* (KIT2005)

Listeria spp., L. monocytogenes

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Listeria* from Swabs
Swabs	Internal validation	1 - 10 mL D/E broth swab	16	1 CFU/swab	1 - 1,000	Add 1 environmental swab to 90 mL of prewarmed (37 °C) 24 LEB Complete media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 35 ± 1 °C for 16 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for Genus *Listeria* or *L. mono*. Utilize SureTrend Quant for result calculations.

Quantification of *Salmonella*

For *Salmonella* - Testing was performed using the BAX® System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* from Swabs
Swabs	Internal validation	1 - 10 mL D/E broth swab	6	1 CFU/mL	1 - 1,000	Add 1 D/E broth environmental swab to BPW media for the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Limits Testing for *Listeria*

For *Listeria* - Testing was performed using the BAX System Real-Time PCR Assay for Genus *Listeria* (KIT2019) -or- the BAX® System Real-Time PCR Assay for *L. mono* (KIT2005)

Listeria spp., L. monocytogenes

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Listeria* from Swabs
Swabs	Internal validation	1 - 10 mL D/E broth swab	16	LOD1	Add 1 environmental swab to 90 mL of prewarmed (37 °C) 24 LEB Complete media as the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 35 ± 1 °C for 16 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for Genus *Listeria* or *L. mono*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Limits Testing for *Salmonella*

For *Salmonella* - Testing was performed using the BAX® System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* from Swabs
Swabs	Internal validation	1 - 10 mL D/E broth swab	6	LOD1	Add 1 D/E broth environmental swab to BPW media for the Primary Enrichment. Homogenize at 230 rpm for 30 seconds. Incubate sample at 42 ± 1 °C for 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize well result interpretation (+/-) to indicate pass or fail for set limit.

Laboratory Isolate Testing

Quantification of *E. coli* O157:H7

For *E. coli* O157:H7 - Testing was performed using the BAX System Real-Time PCR Assay for *E. coli* O157:H7 Exact (KIT2039)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *E. coli* O157:H7 Laboratory Isolates
Pure culture	Internal validation	Overnight culture	0	1,000 CFU/mL	1,000 - 1000,000,000	Create dilutions (1 mL culture into 9 mL BPW) of the overnight culture from 10^{-1} to 10^{-4} of *E. coli*. Choose from any dilution created (10^{-1} to 10^{-4}) and transfer 5 µL into cluster tubes containing lysis buffer to start the PCR process, following manufacturer's instructions for the BAX Real-Time PCR assay for *E. coli* O157:H7 Exact. Utilize SureTrend Quant for result calculations.

Quantification of *Listeria*

For *Listeria* – Testing was performed using the BAX System Real-Time PCR Assay for Genus *Listeria* (KIT2019) -or- the BAX® System Real-Time PCR Assay for *L. mono* (KIT2005)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure - Quantification of *Listeria* Laboratory Isolates
Pure culture	Internal validation	Overnight culture	0	1,000 CFU/mL	1,000-100,000,000	Create dilutions (1 mL culture into 9 mL BPW) of the overnight culture from 10^{-1} to 10^{-4} of Genus *Listeria* or *Listeria monocytogenes*. Select any of these dilutions (10^{-1} to 10^{-4}) and transfer 5 µL into cluster tubes containing lysis buffer to start the PCR process, following the manufacturer's instructions for the BAX Real-Time PCR assay for Genus *Listeria* or *L. mono*. Utilize SureTrend Quant for result calculations.

Quantification of *Salmonella*

For *Salmonella* – Testing was performed using the BAX System Real-Time PCR Assay for *Salmonella* (KIT2006)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure - Quantification of *Salmonella* Laboratory Isolates
Pure culture	Internal validation	Overnight culture	0	1,000 CFU/mL	10,000 - 100,000,000	Create dilutions (1 mL culture into 9 mL BPW) of the overnight culture from 10^{-1} to 10^{-4} of *Salmonella*. Choose from any dilution created and transfer 5 µL into cluster tubes containing lysis buffer to start the PCR process, following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Utilize SureTrend Quant for result calculations.

Quantification of *Vibrio*

For *Vibrio parahaemolyticus, V. vulnificus, V. cholerae* - Testing was performed using the BAX System Real-Time PCR Assay for *Vibrio* (KIT2010)

Sample Type	Validation	Sample Size (g)	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure - Quantification of *Vibrio* Laboratory Isolates
Pure culture	Internal validation	Overnight culture	0	1,000 CFU/mL	1,000-100,000,000	Create dilutions (1 mL culture into 9 mL BPW) of the overnight culture from 10^{-1} to 10^{-4} of *Vibrio*. Choose from any dilution created and transfer 5 µL into cluster tubes containing lysis buffer to start the PCR process, following manufacturer's instructions for the BAX Real-Time PCR assay for *Vibrio*. Utilize SureTrend Quant for result calculations.