Quantification and Limits Manual

Instructions for Specific Matrices and Pathogen Targets Using the BAX® System

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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 to 8 °C
KIT2005	BAX System Real-Time PCR Assay for *L. monocytogenes* (96 tests)	2 to 8 °C
KIT2006	BAX System Real-Time PCR Assay for *Salmonella* (96 tests)	2 to 8 °C
KIT2010	BAX System Real-Time PCR Assay for *Vibrio cholerae/parahaemolyticus/vulnificus* (96 tests)	2 to 8 °C
KIT2018	BAX System Real-Time PCR Assay for *Campylobacter jejuni/coli/lari* (96 tests)	2 to 8 °C
KIT2019	BAX System Real-Time PCR Assay for Genus *Listeria* (96 tests)	2 to 8 °C
MED2010	Buffered Peptone Water (2.5 kg)	2 to 30 °C
MED2003 MED2016 MED2029	BAX System MP Media (2.5 kg, 10 kg, STAT packs)	10 to 25 °C
MED2032	BAX System Quant Solution (25 mL)	2 to 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 Premium (SaaS))	Specifications available at www.hygiena.com
MT-S100	MicroTally® Swabs (or similar)	Purchase from vendor directly

Notes:

See Appendix D for additional information on solution storage, material handling and disposal.

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

For information about using SureTrend® Quant, see videos and instructions accessible in your SureTrend software and instructions in www.hygiena.com/documents.

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 prewarmed (42 °C) BAX MP media containing 1 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*. Use SureTrend Quant for result calculations.
Ceca	Internal validation	1 cecal tonsil	10	10 CFU/mL	10 – 10,000	Add 1 cecal tonsil to 100 mL of prewarmed (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 prewarmed (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*. Use SureTrend Quant for result calculations.
Cloacal Swab	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 prewarmed (42 °C) BAX MP media containing 1 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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (42 °C) BAX MP media containing 8 mg/L of vancomycin. 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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
Feet Swab	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 prewarmed (42 °C) BAX MP media containing 1 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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
Lung	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (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*. Use SureTrend Quant for result calculations.
Trailer Drag Swab	Internal validation	1 Micro-Tally swab	8	10 CFU/mL	10 – 10,000	Wipe 1 MicroTally swab on a poultry trailer and add 200 mL of prewarmed (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*. Use SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
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. Hand massage for 30 seconds for homogenization. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
Carcass Swab	Internal validation	1 carcass swab	6	1 CFU/mL	1 – 10,000	Add 1 carcass swab to 50 mL of prewarmed (42 °C) BPW media as Primary Enrichment. Hand massage for 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*. Use SureTrend Quant for result calculations.
Rinsate	AOAC RI *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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 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*. Use 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 1:4	Internal validation	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
Ground Chicken 1:6	AOAC RI *PTM* 081201	325 g	8	1 CFU/g	1 – 1,000	Add 325 g of comminuted chicken 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 1:1	Internal validation	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
Ground Turkey 1:4	Internal validation	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
Ground Turkey 1:6	AOAC RI *PTM* 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
HPP† Ground Turkey 1:6 (Low Level)	Internal validation	325 g	10	0.1 CFU/g	0.1 – 100	Add 325 g of comminuted turkey to 1,625 mL (1:6) of BAX MP as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds. 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*. Use SureTrend Quant for result calculations.

† HPP = High-Pressure Processing

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Poultry
Chicken Breast	Internal validation	325 g	8	1 CFU/g	1 – 10,000	Add 325 g of raw chicken breast to 1,625 mL (1:6) of prewarmed (35 °C) BPW. Hand massage for 30 seconds for homogenization. Incubate samples at 35 ± 2 °C for 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use SureTrend Quant for result calculations.
		30 mL pour off	6	1 CFU/mL	1 – 10,000	Add 325 g of raw chicken breast to 1,625 mL (1:6) of prewarmed (35 °C) BPW as the Primary Enrichment. Hand massage for 30 seconds for homogenization. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of prewarmed (42 °C) BAX MP. Hand massage for 30 seconds for homogenization. Incubate samples 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*. Use SureTrend Quant for result calculations.
Breaded Stuffed Raw Chicken	Internal validation	375 g	6	1 CFU/g	1 – 10,000	Add 375 g of breaded and stuffed raw chicken product to 375 mL of prewarmed (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 prewarmed (42 °C) BAX MP media containing 1 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*. Use 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	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 prewarmed (42 °C) BAX MP media containing 1 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Ceca	Internal validation	1 cecal tonsil	10	LOD10	Add 1 cecal tonsil to 100 mL of prewarmed (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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Cloacal Swab	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 prewarmed (42 °C) BAX MP media containing 1 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
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 prewarmed (42 °C) BAX MP media containing 8 mg/L of vancomycin. 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Poultry 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Feet Swab	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 prewarmed (42 °C) BAX MP media containing 1 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Lung	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Carcass Swab	Internal validation	1 carcass swab	6	LOD1	Add 1 carcass swab to 50 mL of prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h for 1 – 3 CFU/30 mL enumerable range. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Chicken 1:4	Internal validation	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Chicken 1:6	Internal validation	325 g	8	LOD1	Add 325 g of comminuted chicken 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Ground Turkey 1:1	Internal validation	325 g	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
			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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Turkey 1:4	Internal validation	325 g	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Ground Turkey 1:6	Internal validation	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Chicken Skin 1:3	Internal validation	30 mL pour off	5	LOD1	Add 325 g of chicken skin to 650 mL (1:3) of prewarmed (45 °C) BPW as the Primary Enrichment. Hand massage for 30 seconds for homogenization. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of prewarmed (45 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 30 seconds for homogenization. Incubate samples at 42± 1 °C for 5 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Chicken Skin 1:4	Internal validation	325 g	6	LOD1	Add 325 g of chicken skin to 975 mL (1:4) of prewarmed (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 6 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Chicken Breast 1:3	Internal validation	30 mL pour off	5	LOD1	Add 325 g of raw chicken breast to 650 mL (1:3) of prewarmed (45 °C) BPW as the Primary Enrichment. Hand massage for 30 seconds for homogenization. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of prewarmed (45 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 30 seconds for homogenization. Incubate samples at 42± 1 °C for 5 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Chicken Breast 1:4	Internal validation	325 g	7	LOD1	Add 325 g of raw chicken breast to 975 mL (1:4) of prewarmed (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 7 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
Chicken Breast 1:6	Internal validation	325 g	8	LOD1	Add 325 g of raw chicken breast to 1,625 mL (1:6) of prewarmed (35 °C) BPW. Hand massage for 30 seconds for homogenization. Incubate samples 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
		30 mL pour off	6	LOD1	Add 325 g of raw chicken breast to 1,625 mL (1:6) of prewarmed (35 °C) BPW as the Primary Enrichment. Hand massage for 30 seconds for homogenization. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of prewarmed (42 °C) BAX MP. Hand massage for 30 seconds for homogenization. Incubate samples 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
		30 mL pour off	5	LOD10	Add 325 g of raw chicken breast to 1,625 mL (1:6) of prewarmed (35 °C) BPW as the Primary Enrichment. Hand massage for 30 seconds for homogenization. Transfer 30 mL of the Primary Enrichment into a sterile container with 30 mL of prewarmed (42 °C) BAX MP. Hand massage for 30 seconds for homogenization. Incubate samples 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Breaded Stuffed Raw Chicken	Internal validation	375 g	6	LOD1	Add 375 g of breaded and stuffed raw chicken product to 375 mL of prewarmed (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 prewarmed (42 °C) BAX MP media containing 1 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Poultry
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 prewarmed (42 °C) BPW. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Turkey Thigh	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 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Turkey Wing	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 prewarmed (42 °C) BPW. Hand massage for 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*. Use 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	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 prewarmed (42 °C) 2X Bolton's Broth + 2X supplement (in a sterile, sealable bag). Hand massage for 30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 20 h in microaerophilic conditions (in an air-tight container with microaerophilic gas sachets). Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Use SureTrend Quant for result calculations.
Chicken Neck Skin	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 prewarmed (42 °C) 2X Bolton's Broth + 2X supplement (in a sterile, sealable bag). Hand massage for 30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 16 h under microaerophilic conditions (in an air-tight container with microaerophilic gas sachets). Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Use 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	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 prewarmed (45 °C) 2X Bolton's Broth with 2X supplement (in a sterile, sealable bag). Hand massage for 30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 20 h in microaerophilic conditions (in an air-tight container with microaerophilic gas sachets). Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Chicken Neck Skin	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 prewarmed (45 °C) 2X Bolton's Broth with 2X supplement (in a sterile, sealable bag). Hand massage for 30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 16 h under microaerophilic conditions (in an air-tight container with microaerophilic gas sachets). Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Campylobacter*. Use 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	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantification 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (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 prewarmed (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 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use SureTrend Quant for result calculations.
Feces (High Level)	Internal validation	10 g	–	100,000 CFU/g	100,000 – 100,000,000	Add 10 g of beef feces to 90 mL of prewarmed (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 prewarmed (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*. Use SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantification of *Salmonella* in Beef
MicroTally Drain Swab	Internal validation	1 MicroTally swab	6	1 CFU/mL	1 – 10,000	Add 1 MicroTally to 200 mL of prewarmed (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*. Use 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 prewarmed (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*. Use 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 prewarmed (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 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use SureTrend Quant for result calculations.
Cecal Contents (High Level)	Internal validation	10 g	–	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 prewarmed (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*. Use SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantification 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 prewarmed (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*. Use SureTrend Quant for result calculations.
Lymph Node	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 prewarmed (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*. Use SureTrend Quant for result calculations.
		>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 prewarmed (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*. Use SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure- Quantification of *Salmonella* in Beef
MicroTally (Trim)	AOAC RI *PTM* 081201	1 MicroTally swab	6	1 CFU/mL	1 – 10,000	Add 1 MicroTally (swabbed on beef trim) to 200 mL of prewarmed (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*. Use SureTrend Quant for result calculations.
Trim	AOAC RI *PTM* 081201	375 g	6	1 CFU/g	1 – 10,000	Add 375 g of beef trim to 1,500 mL of prewarmed (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*. Use SureTrend Quant for result calculations.
Ground Beef	AOAC RI *PTM* 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 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*. Use 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	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed 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 prewarmed (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 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Feces (High Level)	Internal validation	10 g	–	LOD 100,000	Add 10 g of beef feces to 90 mL of prewarmed 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 prewarmed (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*. Use 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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	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 prewarmed (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 8 h. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Cecal Contents (High Level)	Internal validation	10 g	–	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 prewarmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage 30 seconds for homogenization. Test immediately – proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use 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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Lymph Node	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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
		> 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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Beef
MicroTally (Trim)	Internal validation	1 MicroTally swab	4	LOD10	Add 1 MicroTally (swabbed on beef trim) to 200 mL of prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
			6	LOD1	Add 1 MicroTally (swabbed on beef trim) to 200 mL of prewarmed (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*. Use 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 prewarmed (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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 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*. Use 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	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 prewarmed (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. Use 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	Incubation Time (h)	Limits LOD**	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 prewarmed (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. Use 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	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (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 prewarmed (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*. Use SureTrend Quant for result calculations.
Feces (High Level)	Internal validation	10 g	–	100,000 CFU/g	100,000 – 100,000,000	Add 10 g of pork feces to 90 mL of prewarmed (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 prewarmed (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*. Use SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Pork
Cecal Swab (High Level)	Internal validation	1 – 25 mL pre-moistened BPW swab	–	100,000 CFU/mL	100,000 – 100,000,000	Swab 1 pork ceca with a 25 mL pre-moistened BPW swab and combine with 50 mL of prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds. Test immediately for 100,000 – 100,000,000 CFU/g enumerable range.
Rope	Internal validation	1 rope	6	100 CFU/mL	100 – 10,000	Add 1 pork rope 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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. Use SureTrend Quant for result calculations.
Spleen	Internal validation	25 g	8	1 CFU/g	1 – 10,000	Add 25 g of pork 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
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*. Use SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Pork
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 prewarmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.
Lymph Node	Internal validation	<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 prewarmed (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*. Use SureTrend Quant for result calculations.
		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 prewarmed (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*. Use SureTrend Quant for result calculations.
Ground Pork	AOAC RI *PTM* 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 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*. Use SureTrend Quant for result calculations.

Sample Type	Validation	Sample Size	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Salmonella* in Pork
MicroTally (Trim)	AOAC RI *PTM* 081201	1 MicroTally swab	6	1 CFU/mL	1 – 10,000	Add 1 MicroTally (swabbed on pork trim) to 200 mL of prewarmed (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*. Use SureTrend Quant for result calculations.
Trim	AOAC RI *PTM* 081201	375 g	6	1 CFU/g	1 – 10,000	Add 375 g of pork trim to 1,500 mL of prewarmed (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*. Use 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	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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 prewarmed (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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Feces (High Level)	Internal validation	10 g	–	LOD 100,000	Add 10 g of pork feces to 90 mL of prewarmed (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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Pork
Cecal Swab (High Level)	Internal validation	1 – 25 mL pre-moistened BPW swab	–	LOD100,000	Swab 1 pork ceca with a 25 mL pre-moistened BPW swab and combine with 50 mL of prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds. Test immediately – proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Rope	Internal validation	1 rope	6	LOD100	Add 1 pork rope 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Spleen	Internal validation	25 g	8	LOD1	Add 25 g of pork 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 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*. Use 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	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 prewarmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
Lymph Node	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 prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
		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 prewarmed (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*. Use 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 prewarmed (42 °C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Sample Type	Validation	Sample Size	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* in Pork
MicroTally (Trim)	Internal validation	1 MicroTally swab	4	LOD10	Add 1 MicroTally (swabbed on pork trim) to 200 mL of prewarmed (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*. Use 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 prewarmed (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*. Use 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	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 25 g of oyster meat to 250 mL of prewarmed (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*. Use 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	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Vibrio* in Seafood
Oysters	Internal validation	25 g	6	LOD10	Add 25 g of oyster meat to 250 mL of prewarmed (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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.
			8	LOD1	Add 25 g of oyster meat to 250 mL of prewarmed (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 for LOD1. Following incubation, proceed to PCR testing following manufacturer's instructions for the BAX Real-Time PCR assay for *Vibrio*. Use 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	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 (35 °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*. Use 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	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 (35 °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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Environmental Monitoring 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* from Swabs
Swab	Internal validation	1 – 10 mL D/E broth swab	6	1 CFU/mL	1 – 1,000	Add 1 D/E broth environmental swab to 50 mL of 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*. Use 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	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Salmonella* from Swabs
Swab	Internal validation	1 – 10 mL D/E broth swab	6	LOD1	Add 1 D/E broth environmental swab to 50 mL 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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

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	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Listeria* from Swabs
Swab	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 (35 °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*. Use 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	Incubation Time (h)	Limits LOD**	Procedure – Limits Testing for *Listeria* from Swabs
Swab	Internal validation	1 – 10 mL D/E broth swab	16	LOD1	Add 1 D/E broth environmental swab to 90 mL of prewarmed (35 °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*. Use well result interpretation (+/-) to indicate pass or fail for set limit.

Laboratory Isolate Testing

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	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *E. coli* O157:H7 Laboratory Isolates
Pure Culture	Internal validation	Overnight culture	–	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 20 µL into cluster tubes containing lysis reagent (buffer + protease) to start the PCR process, following manufacturer's instructions for the BAX Real-Time PCR assay for *E. coli* O157:H7 Exact. Use SureTrend Quant for result calculations.

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	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Listeria* Laboratory Isolates
Pure Culture	Internal validation	Overnight culture	–	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 reagent (buffer + protease + Lysing Agent 2) to start the PCR process, following the manufacturer's instructions for the BAX Real-Time PCR assay for Genus *Listeria* or *L. monocytogenes*. Use SureTrend Quant for result calculations.

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* Laboratory Isolates
Pure Culture	Internal validation	Overnight culture	–	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 (buffer + protease) to start the PCR process, following manufacturer's instructions for the BAX Real-Time PCR assay for *Salmonella*. Use SureTrend Quant for result calculations.

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	Incubation Time (h)	mLOQ*	Enumerable Range (CFU)	Procedure – Quantification of *Vibrio* Laboratory Isolates
Pure Culture	Internal validation	Overnight culture	–	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 (buffer + protease) to start the PCR process, following manufacturer's instructions for the BAX Real-Time PCR assay for *Vibrio*. Use SureTrend Quant for result calculations.

Appendix A: BAX System Quant Evaluation Method

Overview

BAX® System Quant (BAX Quant) is a rapid quantification method that uses the cycle threshold (Ct) values from the BAX System to enumerate the level of pathogens (*Salmonella* spp.*, Campylobacter* spp.*,* Genus *Listeria, Listeria monocytogenes, E. coli* O157:H7 and *Vibrio* spp.) from food products, environmental surfaces or laboratory samples. The SalQuant® workflows have been certified by AOAC International for several matrices (Table 1).

Table 1. Overview of BAX System SalQuant AOAC Certification.

Validated Matrices for BAX System SalQuant	Sample Size	AOAC Certification
Comminuted Chicken Comminuted Turkey Poultry Rinsates	325 g 325 g 30 mL	AOAC RI *PTM*℠ 081201
Ground Beef Beef Trim MicroTally®, Beef Trim	375 g 375 g 1 cloth	
Ground Pork Pork Trim MicroTally, Pork Trim	375 g 375 g 1 cloth	

Regulatory guidelines recommend user verification of candidate methods; the best practice is to perform a paired study design using a common enriched test portion that is analyzed by both the BAX Quant method and the reference method to ensure optimal performance. Verification studies are intended to demonstrate that validated methods perform according to the method specifications determined in the validation study when in the user's hand and show that the candidate method is fit for purpose.

Aim

The aim of this document is to provide an evaluation that compares the performance of BAX Quant to a known reference method. The paired study design is used to remove as many differences as possible by running a side-by-side comparison of BAX Quant to the most probable number (MPN) technique or another quantitative platform.

Results from BAX Quant and the comparison method can be entered into a data analysis spreadsheet (*BAX Quant Evaluation Spreadsheet*) to statistically assess the performance of the two methods [1].

Study Design

Sample Selection
Select representative product samples and/or locations from your facility. Based on the sample selected, use the appropriate quantification protocol from this manual.

Quantitative Study
For each matrix, test a minimum of 30 samples with contamination levels representative of the natural contamination seen in the sample type tested. If samples are required to be inoculated, at least two desired target levels should be spiked with at least one uninoculated sample. See additional recommendations in the "Culture" section below.

Required Equipment

- BAX System Q7 Start-up Package (equipment and supplies for 192 initial tests), Product No. ASY2018 (120 V) or ASY2020 (220 V)
- Incubator set to the proper temperature (35 °C or 42 °C)
- Reference method or comparison platform

Consumables
Use the methods and materials described in this manual for your required test parameters. Quantification methods have been developed for the following Hygiena® kits:

BAX System Real-Time PCR Assays	Product No.
Campylobacter jejuni, C. coli, C. lari	KIT2018
E. coli O157:H7 Exact	KIT2039
Genus *Listeria*	KIT2019
Listeria monocytogenes	KIT2005
Salmonella	KIT2006
Vibrio cholera, V. parahaemolyticus, V. vulnificus	KIT2010

Culture
Grow pure cultures based on internal SOP. For consistency, it is recommended that you select a strain used in relevant certification reports.

Testing Notes

- Ensure the media is pre-warmed, if required in the protocol.
- After sample collection and preparation, the media will be split to accommodate MPN and/or plating methods.
- Highly contaminated samples (e.g., matrices that are frequently found contaminated with the pathogen of interest in high quantities) should be serially diluted in the appropriate product sample diluent before testing to ensure comparable results with BAX Quant vs. the reference method (MPN and/or plating methods).

Study Protocol

Sample Preparation and BAX Quantification
Collect samples using aseptic technique and following the appropriate procedure described in this manual for your sample type. In brief:

1. Add sample into a sterile sample bag or collection device.
   Notes:
   - If spiking samples: spike sample directly to and immediately before adding the enrichment media.
   - For spiked liquid samples: after spiking, see “Verification Sample Prep” below to process part of the samples using a reference method.
2. Add the required primary enrichment media.
   Note: For solid samples: after inoculation, see “Verification Sample Prep” below to process part of the samples using a reference method.
3. If applicable for your sample, combine an aliquot with pre-warmed secondary enrichment media.
   Note: If storing samples for hours/days, do not add secondary enrichment media until ready for use.
4. Incubate samples at the required temperature and time.
5. Run samples on the BAX System at the Quantification timepoint.
6. After the BAX run, upload the BAX file to SureTrend® and calculate bacterial culture estimations through SureTrend Quant.
   Note: Estimations will be in Log CFU/mL (g), CFU/mL (g) or Log CFU/sample.

Verification Sample Prep and Quantification
Most Probable Number (MPN)

This method is recommended for *Salmonella* and *E. coli*. In the following modified MPN protocol, incubated MPN tubes are tested using BAX System Real-Time PCR Assays, instead of culture confirmation. Follow the USDA guidelines and reference the MPN documentation for additional details [2].

1. Set up the MPN rack with 3 replicates of 5 sample dilutions, representing 1, 0.1, 0.01, 0.001 and 0.0001 mL of sample (Figure 1).
   a. For the 10^{-1} dilution, add 1 mL of sample (undiluted liquid sample or primary enrichment of the solid sample) to each of the 3 first tubes. To each 1 mL aliquot, add 9 mL BPW.
   b. Perform serial dilutions until the 10^{-4} dilution is reached.
   Note: 15 total MPN tubes are needed per sample tested.
2. Incubate tubes at the temperature and time needed for the strain used.
   For example: For naturally occurring *Salmonella* and *E. coli*, incubate tubes for 20 – 24 h at 35 ± 2 °C (Figure 1).
3. Test each MPN tube on the BAX System (MPN-PCR).
4. Refer to MPN tables for the calculation of MPN/g (or MPN/mL) [2, pages 5 – 8] and record in a spreadsheet.
5. Transform MPN analyzed data to Log CFU/g (or CFU/mL) and input into the Evaluation Spreadsheet [1].

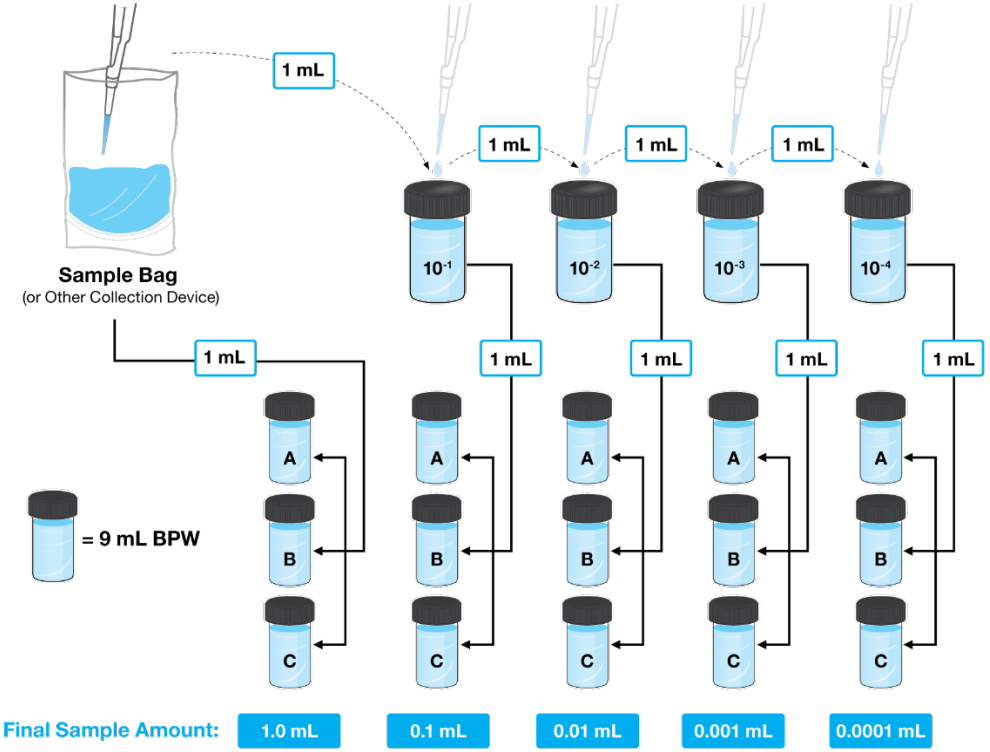

Figure 1. Example Setup of the Most Probable Number Method for Estimating *Escherichia coli* and *Salmonella* in a Sample. There are 3 replicates (A, B and C) of the sample and 4 dilutions (10^{-1} to 10^{-4}), resulting in 15 MPN tubes per sample matrix.

Agar Plating

This method is recommended for *Campylobacter*, *Listeria* and *Vibrio*. Follow the manufacturer's instructions for spreading, streaking and incubating the culture plate.

1. For undiluted liquid samples or the primary enrichment of solid samples, spread 100 µL of sample onto a selective agar plate.
2. Invert plates and incubate at the manufacturer's recommended time and temperature.
3. Count typical colonies and record the number in a spreadsheet.
4. Back calculate CFU values based on dilutions to determine CFU/g or CFU/mL of product.

   For example, plating method counts using a 1:10 dilution (100 µL sample into 900 µL diluent dilution) will be multiplied by 10 to back calculate to CFU/g or CFU/mL.

Data Management

1. Input Quant, MPN and agar plate data into the provided evaluation spreadsheet for analysis.
2. Collect all BAX files in a central folder for later access.

References

1. Hygiena. (2023) BAX Quant Evaluation Spreadsheet. www.hygiena.com/documents.
2. USDA. (2014) USDA-FSIS MLG Most Probable Number (MPN) 2.05.

Appendix B: Quant Solution Preparation

As supplied:

- Store at 2 to 8 °C
- Appearance: colorless/clear solution
- Shelf Life: Refer to the expiration date on the Certificate of Analysis
- Volume: 25 mL bottle

When added to media:

1. Prepare 1 L of BAX® MP media according to manufacturer's specifications.
   Note: Once prepared and autoclaved, BAX MP media is stable for 2 – 4 weeks at 2 to 8 °C.
2. Cool to 45 to 55 °C.
3. Immediately before use, aseptically transfer 0.5 mL or 1.0 mL of Quant Solution (depending on the protocol) to 1 L of cooled BAX MP media (<55 °C) and mix.
4. Use within 24 hours, then discard remaining BAX MP with Quant Solution according to site practices as required by federal, state and local regulations.

Appendix C: Additional Information

Material Handling, Storage and Disposal

Cycler/Detector
The instrument requires a constant supply of air that is 31 °C or cooler to remove heat generated by operation. If the air supply is inadequate or too hot, the machine can overheat, causing performance problems, software error messages and even automatic shutdowns. Please see the guidelines for installation in the BAX® User Guide, Chapter VII: BAX System Hardware.

The cycler/detector can generate enough heat to inflict serious burns and can deliver strong electrical shocks if not used according to the directions in this manual. Please read the safety considerations in the BAX User Guide, Chapter I: BAX System Method Overview before using this instrument for the first time.

Reagents and Supplies
The BAX System method includes sample preparation enrichment procedures that nourish the growth of potential pathogens to detectable levels. Because pathogens can cause human illness, appropriate safety precautions must be taken when handling samples, media, reagents, glassware and other supplies and equipment that could be contaminated with potentially pathogenic bacteria.

Reagents used with the BAX System assays should pose no hazards when used as directed. Before using this product, please review the Safety Data Sheets (SDS), available on Hygiena's website. Refer to your site practices for safe handling of materials at extreme temperatures. www.hygiena.com

Storage

- Reagent packages should be kept refrigerated at 2 to 8 °C. Do not freeze.
- Cooling blocks should be kept refrigerated at 2 to 8 °C and used within 30 minutes of removal from refrigerator.
- After protease has been added to the lysis buffer, the shelf life of the solution is two weeks when stored at 2 to 8 °C.
- If storing PCR tubes with tablets in an open kit for more than 3 weeks, place into a larger bag with desiccant or store at 4 °C in a desiccation unit, if possible.

  ***Note**: Storage of PCR tubes with desiccant is particularly important for real-time assays.*
- Reagents should be used by the expiration date stamped on the individual labels.
- Pipettes should be calibrated to deliver within 10% of required volumes. Barrier tips are recommended for all pipettes.
- Please see the manufacturer's documentation for handling, disposal and storage of the pipettes, computer system and other equipment.

Disposal
Decontaminate materials and dispose of biohazardous waste according to your site practices and as required by federal, state and local regulations.

For additional recommendations about preventing, identifying and removing PCR contamination, see the BAX System Q7 User Guide, Appendix B: PCR, Contamination Control.

Technical Assistance

Global Support
For detailed troubleshooting on the BAX System instrument, see Appendix D of the BAX System Q7 User's Guide. If you have any additional questions or comments on the BAX System or quantification procedures, please contact Hygiena Diagnostics Support directly by email at diagnostics.support@hygiena.com.