hygiena™

Pathogen Detection and Quantification for the Beef Industry

INS2039-REVA

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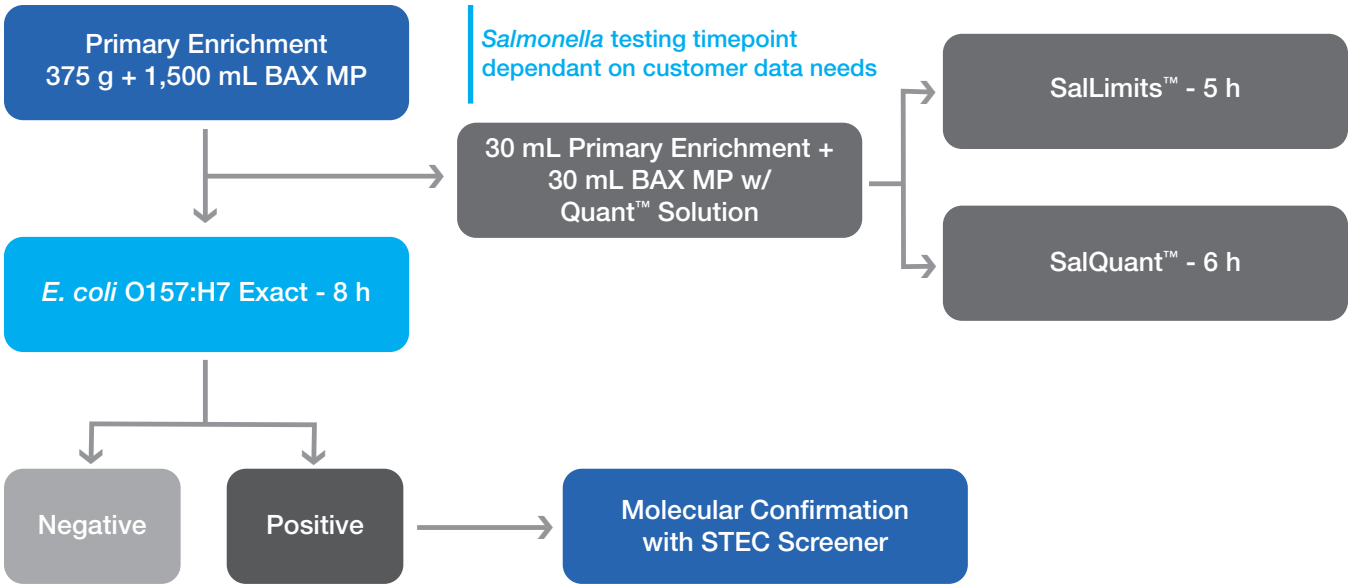
Current Industry Workflows - Beef



Salmonella Detection in Beef

For beef trim, 60 pounds was sourced from a local butcher for sampling. MicroTally™ manual sampling devices (MSD) were removed from the sample bag, unfolded and firmly used to swab the beef trim. Once both sides of the cloth were used, swabs were folded back to the original dimensions with an additional horizontal fold per the manufacturer's instructions and placed into the original sample bag. The MSD swabs (n=15) were then spiked with various levels of *Salmonella* (1-10,000 CFU), homogenized in the proper enrichment medium and incubated. After 3-6 hours, samples were analyzed using the **BAX® System Real-Time PCR Assay for *Salmonella***. All inoculated samples were detected after 4, 5, and 6 hours of enrichment and all 30 were confirmed with culture.

Salmonella Quantitation

When repeated for ground beef and beef trim (and MicroTally MSD swabs) using contamination levels of 10 CFU/g, the **BAX System Real-Time PCR Assay for *Salmonella*** detected the presence of the organism at 4-5 hours of enrichment. In addition, after a 6-hour enrichment, *Salmonella* could be quantified from 1-10,000 CFU/g when following the SalQuant™ approach. This allows beef processors to not only identify the presence of *Salmonella*, but also to identify which lots of ground beef or beef trim contain higher levels of *Salmonella*, allowing for rapid action to reduce risk of exposure to consumers and to improve food safety processes internally. (Similar results were also obtained for poultry.)

BAX System Q7

AOAC Validation Summary

AOAC-RI *PTM*SM Level 2 Modification for Hygiena™ BAX System SalQuant Utilizing BAX System Real-Time PCR Assay for *Salmonella* for 7 Matrix Extensions

Process control and final product decisions based only on prevalence have shown limitations reducing consumer risk. Therefore, adoption of validated quantification methodologies with low error and wide enumerable ranges should be utilized to make data-driven food safety decisions.

This certification provides the poultry, beef, and pork industries with an accurate, reliable, and validated quantification tool to reduce product hold-times, verify corrective actions, monitor process control, and promote faster data-driven diversion decisions which ultimately reduces consumer risk in animal protein products.

Validation Methods

- The evaluation consisted of 7 matrix studies to extend the methods' claims.
- Three distinct levels with a range of 1.0 Log CFU/mL(g) are established by the AOAC committee based upon enumeration capabilities of the candidate method.
- Each level has 5 unpaired individual samples that are tested for each level with the mean and error of each level utilized for comparison to the reference method.
- The candidate method must be within +/- 0.5 Log CFU/mL(g) of the MLG MPN 2.05 reference method for each level and be within the 90% confidence interval.

Matrix	Sample Size	Incubation Conditions	Enumerable Range
Poultry Rinsate	30 mL	42°C for 6 h	1 – 10,000 CFU/mL
Ground Beef	375 g	42°C for 6 h	1 – 10,000 CFU/g
Ground Pork	375 g	42°C for 6 h	1 – 10,000 CFU/g
Beef Trim	375 g	42°C for 6 h	1 – 10,000 CFU/g
Pork Trim	375 g	42°C for 6 h	1 – 10,000 CFU/g
MicroTally - Beef Trim	1 Swab	42°C for 6 h	1 – 10,000 CFU/mL
MicroTally - Pork Trim	1 Swab	42°C for 6 h	1 – 10,000 CFU/mL

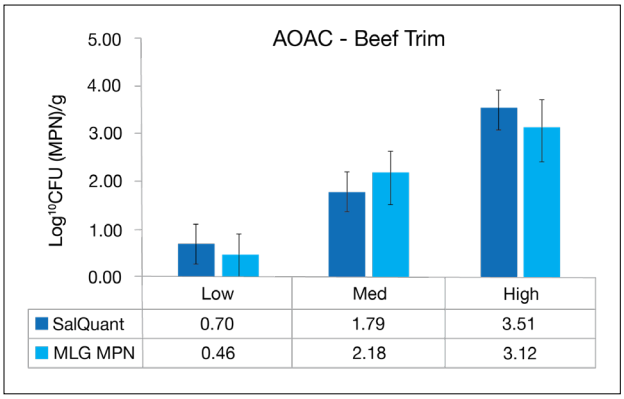
Validation Results

- The Level 2 modification to the **BAX System Real-Time PCR Assay for *Salmonella*, BAX System SalQuant** (Certification No. 081201) was evaluated and approved by the AOAC Research Institute *Performance Tested Methods*^{SM} Program on January 12, 2022.
- Results of the validation study showed the SalQuant demonstrated comparable performance to that of the USDA-FSIS MPN reference methods for estimating *Salmonella* spp.

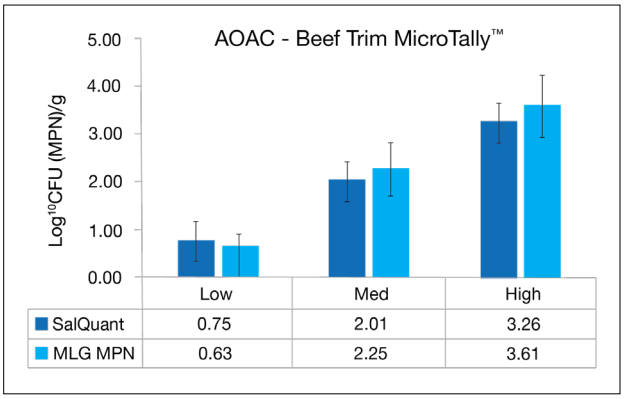
Application Highlights

- **One enrichment, one sample prep, one assay;** no additional equipment, consumables, or steps.
- **Widest enumerable range** across all matrices to facilitate contamination levels observed in sample types taken from farm to final product.
- **Lowest level of enumeration** (1 CFU/g(mL)) in order to truly quantify consumer risk.
- **Largest data generation (>100,000 tests)** to develop, verify, and validate on real industry samples across matrices, locations, facilities, instruments, and users for robustness and integrity of results.

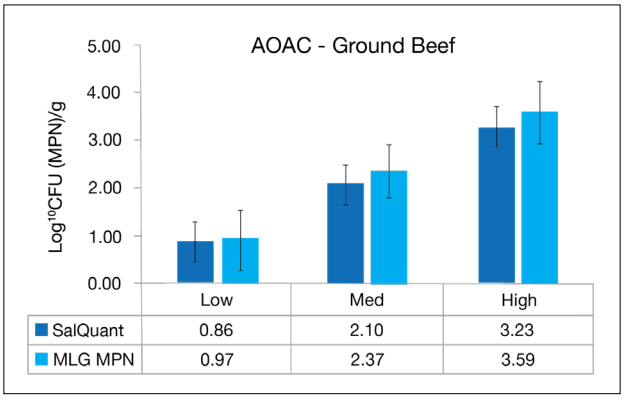
AOAC-RI *PTM*^{SM} Level 2 Modification for Hygiena BAX System SalQuant Utilizing BAX System Real-Time PCR Assay for *Salmonella* for Beef Matrix Extensions



	Low	Med	High
SalQuant	0.70	1.79	3.51
MLG MPN	0.46	2.18	3.12

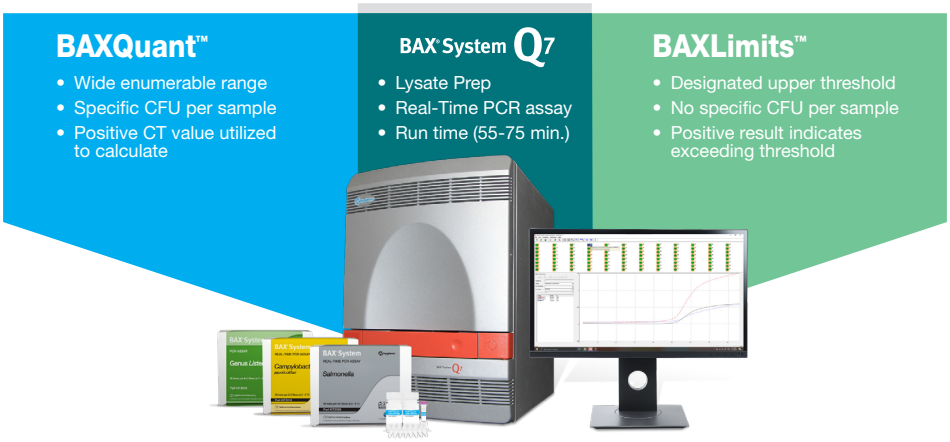


	Low	Med	High
SalQuant	0.75	2.01	3.26
MLG MPN	0.63	2.25	3.61



	Low	Med	High
SalQuant	0.86	2.10	3.23
MLG MPN	0.97	2.37	3.59

Quantification Options



Limit of Quantification (LOQ)

If SalQuant samples are negative, but positive at prevalence, the result should be ≤ enumerable range. (i.e., a poultry rinse was negative at the 6 h timepoint. The sample underwent continued incubation and was tested for prevalence. The prevalence test was positive; therefore with a negative quantification test, but a positive prevalence test, the result for quantification would be < 1 CFU/mL).

Application	Industry	Segment	Matrix	Tiimepoint	Limit of Quantification
SalQuant	Beef	Primary Production	Feces	8 h	10 CFU/g
SalQuant	Beef	Primary Production	Feces (high*)	0 h	100,000 CFU/g
SalQuant	Beef	Processing	Lymph Nodes (small)	6 h	10 CFU/Lymph Nodes
SalQuant	Beef	Processing	Lymph Nodes (medium)	6 h	10 CFU/Lymph Nodes
SalQuant	Beef	Processing	Cecal Swabs	8 h	10 CFU/mL
SalQuant	Beef	Processing	Cecal Contents	8 h	1 CFU/g
SalQuant	Beef	Processing	Cecal Contents (high*)	0 h	100,000 CFU/g
SalQuant	Beef	Final Product	Ground Beef	6 h	1 CFU/g
SalQuant	Beef	Final Product	Trim	6 h	1 CFU/g
SalQuant	Beef	Final Product	MicroTally	6 h	1 CFU/mL
SalQuant	Environmental	Environmental	Swabs	6 h	1 CFU/mL

* high = high concentration of *Salmonella*

Limit of Detection (LOD)

LOD is utilized as a limits approach or threshold testing. No calculations are utilized to determine LOD, only the timepoint and detection of bacteria indicate the limit of detection has been met. (i. e., ground beef LOD at 5 h is 10 CFU, therefore if the sample is positive at 5 hours, the results would be ≥ 10 CFU/g)

Application	Industry	Segment	Matrix	Tiimepoint	Limit of Detection
SalQuant	Beef	Final Product	Ground Beef	5 h	≥ 10 CFU/g
SalQuant	Beef	Final Product	Trim	4 h	≥ 10 CFU/g
SalQuant	Beef	Final Product	MicroTally	4 h	≥ 10 CFU/mL

Beef
Primary Production

Feces
Enrichment & PCR Procedure

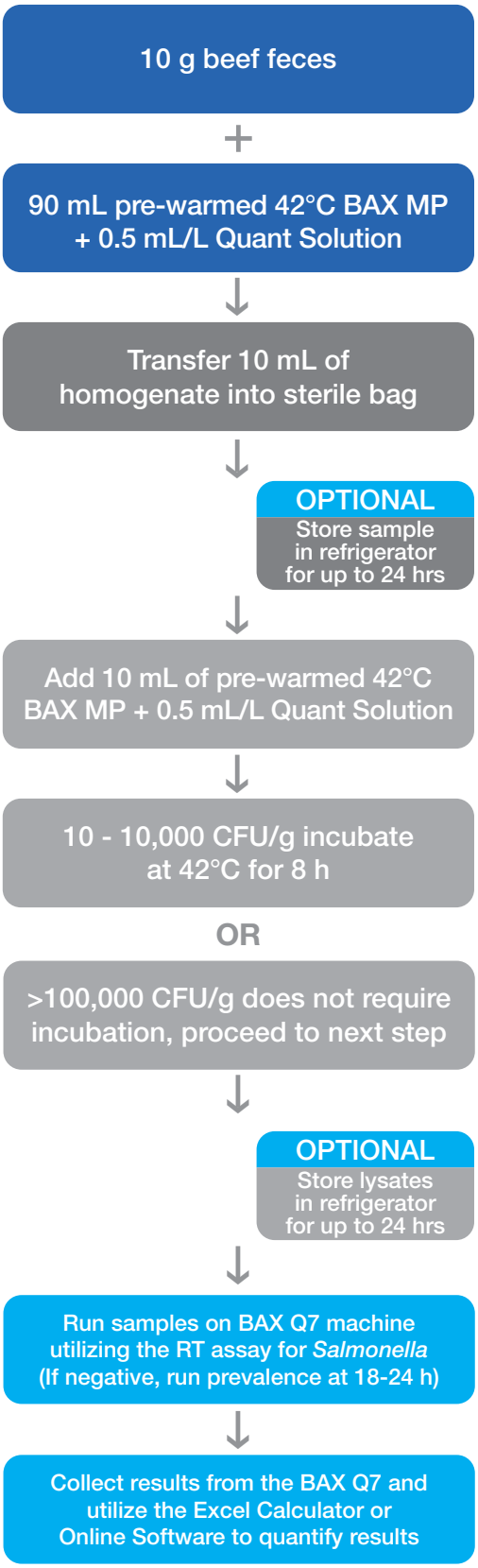
Add 10 g of beef feces to 90 mL of pre-warmed 42°C BAX MP + 0.5 mL/L Quant Solution as primary enrichment. Homogenize by hand for 60 seconds.

Transfer 10 mL of primary enrichment into a sterile container with 30 mL of pre-warmed (42°C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization.

Samples with enumerable ranges from 10 - 10,000 CFU/g require incubation at 42°C for 8 h. Samples with greater than 100,000 CFU/g do not require incubation, proceed directly to PCR.

Following enrichment for quantification, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel® Calculator or Online Software to quantify results.

After transferring aliquot for quantification enrichment, incubate the remaining sample in primary enrichment at 37 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef Processing

Lymph Nodes Enrichment & PCR Procedure

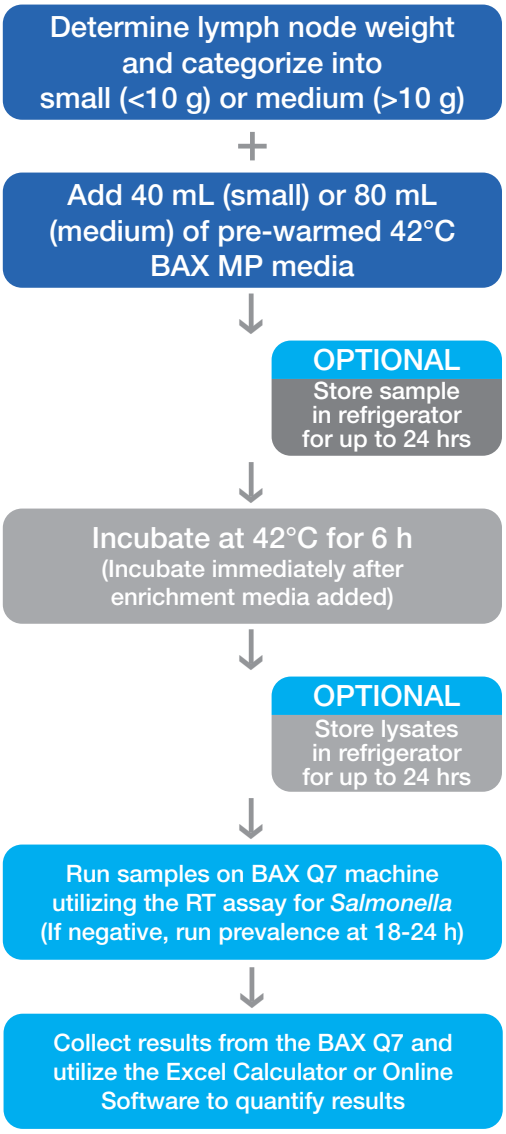
Weigh and process lymph nodes into small (<10 g) or medium (>10 g) size category.

For small nodes, add 40 mL of pre-warmed (42°C) BAX MP media and for medium nodes, 80 mL of pre-warmed (42°C) BAX MP media as primary enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1°C for 6 h.

Following enrichment for quantification or LOD, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results.

After quantification enrichment, incubate the remaining primary enrichment at 37 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef Processing

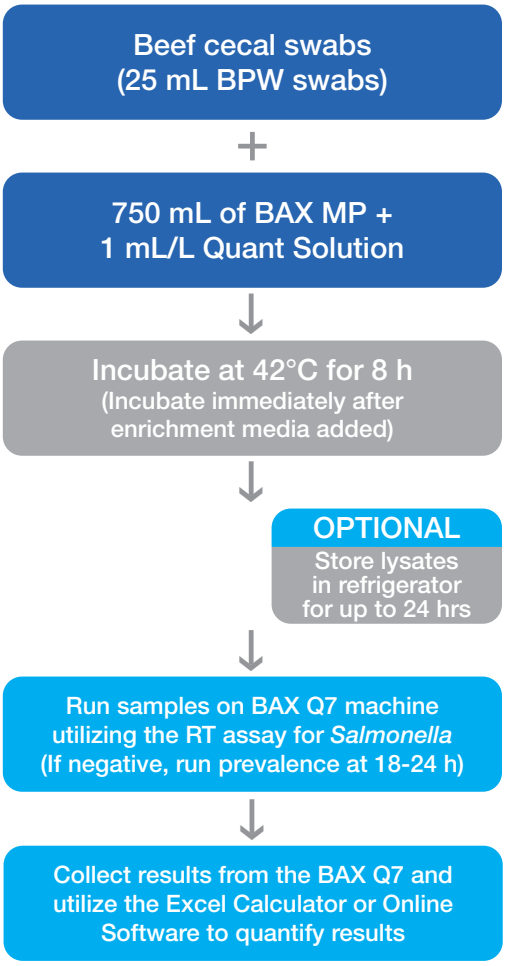
Cecal Swabs Enrichment & PCR Procedure

Swab beef ceca with a 25 mL pre-moistened BPW swab and combine with 50 mL of pre-warmed (42°C) BAX MP media containing 1 mL/L of BAX Quant Solution as the primary enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1°C for 8 h.

Following enrichment for quantification, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results.

After quantification enrichment, incubate the remaining primary enrichment at 42 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef Processing

Cecal Contents Enrichment & PCR Procedure

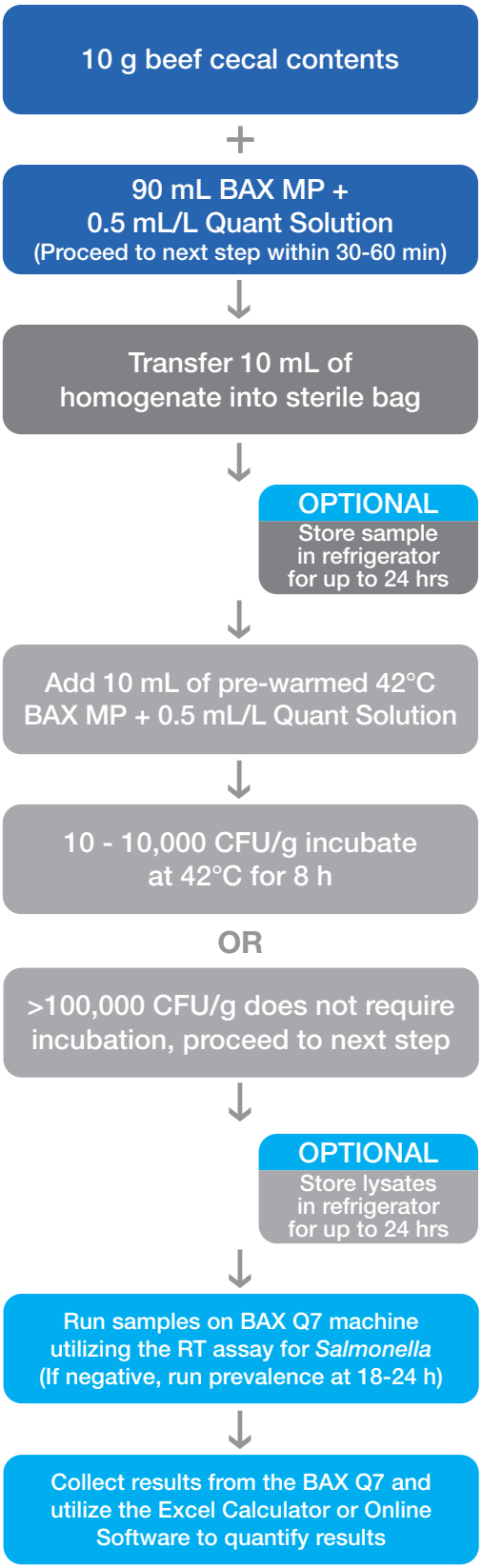
Add 10 g of beef cecal contents to 90 mL of BAX MP with + 0.5 mL/L Quant Solution as the primary enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 10 mL of the primary enrichment into a sterile container with 10 mL of pre-warmed (42°C) BAX MP media containing 0.5 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization.

Samples with enumerable ranges from 10 - 10,000 CFU/g require incubation at 42°C for 8 h. Samples with greater than 100,000 CFU/g do not require incubation, proceed directly to PCR.

Following enrichment for quantification, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results.

After transferring aliquot for quantification enrichment, incubate the remaining primary enrichment at 37 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef Processing

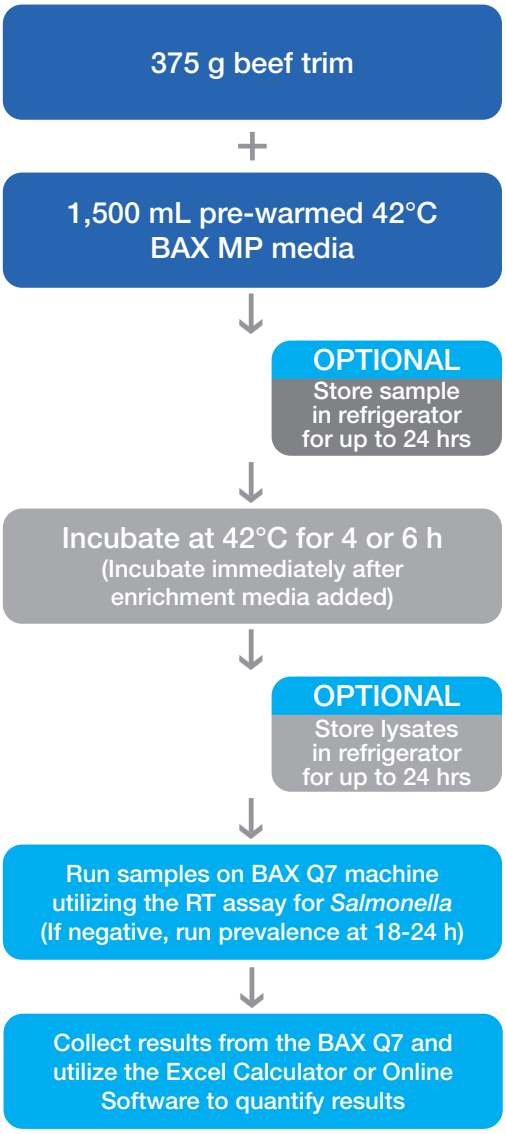
Trim Enrichment & PCR Procedure

Add 375 g of beef trim to 1,500 mL of pre-warmed (42°C) BAX MP media as primary enrichment. Homogenize at 230 RPM for 30 seconds

Incubate sample at 42 ± 1°C for 4 h for LOD10 or 6 h for LOD1 and 1 - 10,000 CFU/g enumerable range.

Following enrichment for quantification or LOD, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results.

After quantification enrichment, incubate the remaining primary enrichment at 37 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef Processing

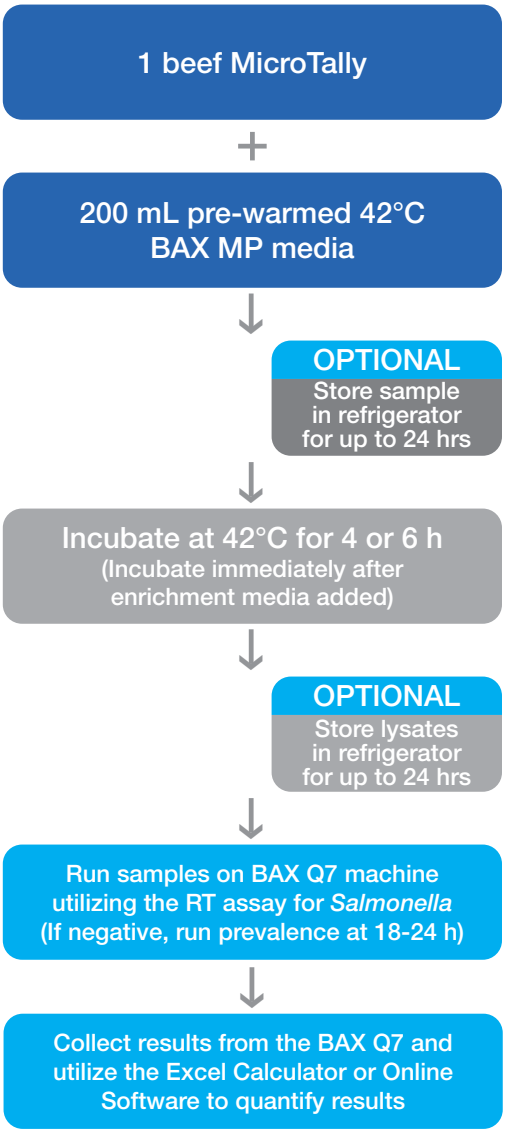
MicroTally Enrichment & PCR Procedure

Add 1 MicroTally to 200 mL of pre-warmed (42°C) BAX MP media as primary enrichment. Homogenize at 230 RPM for 30 seconds

Incubate sample at 42 ± 1°C for 4 h for LOD10 or 6 h for LOD1 and 1 - 10,000 CFU/g enumerable range.

Following enrichment for quantification or LOD, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results.

After quantification enrichment, incubate the remaining primary enrichment at 37 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef
Final Product

Ground Beef
Enrichment & PCR Procedure

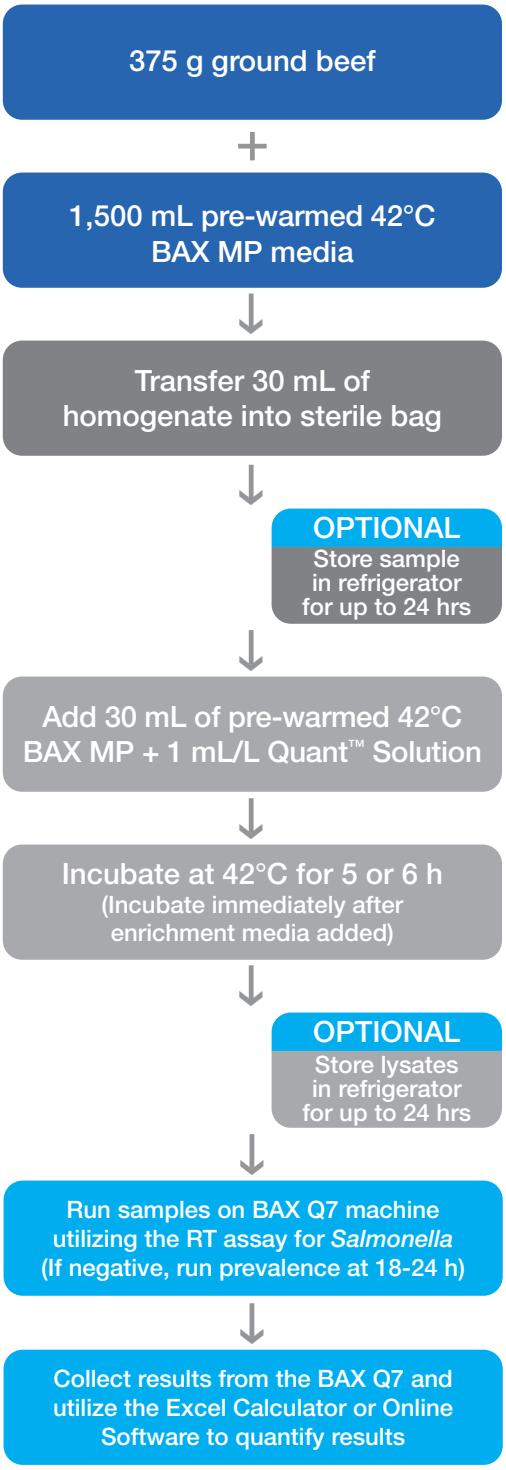
Add 375 g of ground beef to 1,500 mL of pre-warmed (42°C) BAX MP media as primary enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 30 mL of primary enrichment into a sterile container with 30 mL of pre-warmed (42°C) BAX MP media containing 1 mL/L of Quant Solution. Hand massage for 15-30 seconds for homogenization.

Incubate sample at 42 ± 1°C for 5 h for LOD10 or 6 h for LOD1 and 1 - 10,000 CFU/g enumerable range.

Following enrichment for quantification or LOD, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results.

After transferring aliquot for quantification enrichment, incubate the remaining primary enrichment at 37 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Environmental Monitoring

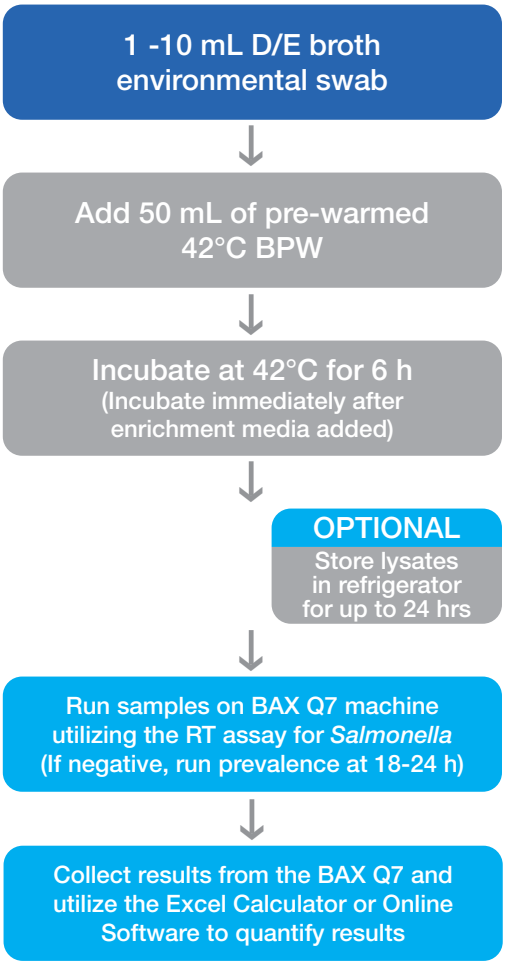
Swab - D/E Broth Enrichment & PCR Procedure

Add 1 environmental swab to 50 mL of pre-warmed (42°C) BPW media as the primary enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1°C for 6 h.

Following enrichment for quantification, perform PCR analysis with the BAX System Real-Time PCR Assay for *Salmonella*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results.

After quantification enrichment, incubate the remaining primary enrichment at 42 ± 1°C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Quantification Workflow Comparison Beef Trim

BAX® System SalQuant™	GENE-UP® Quant *Salmonella*
375 g beef trim	100 g beef trim
1,500 mL BAX MP	5X PBS or other media
Homogenize/stomach	Homogenize/stomach
Incubation at 42°C for 5 – 6 h	No incubation
Transfer 5 µL into lysis solution	Transfer 40 mL of sample into 50 mL tube
Heat at 37°C for 10 min	Centrifuge for 10 min into a 500 g pellet of debris
Heat at 95°C for 20 min	Transfer 25 mL of supernatant to clean tube
Cool in cold block for 5 min	Centrifuge for 10 min at 4300 g, to concentrate *Salmonella*
Hydrate BAX System Real-Time PCR Assay *Salmonella* with 30 µL of lysate	Decant supernatant
Initialize and run the BAX System (75 min)	Resuspend pellet with 600 µL of Promega Nuclei Lysis Solution
Utilize the BAX Cycle Threshold (CT) in Excel spreadsheet or BAXQuant Online Software	Vortex for 10 seconds
True quantification results available	Transfer 600 µL of sample into 1.5 mL tube
	Incubate at 80°C for 5 min
	Cool on ice for 20 min
	Resuspend pellet with 600 µL of Promega Nuclei Lysis Solution
	Add 200 µL of Promega Protein Precipitation Buffer
	Vortex sample solution
	Incubate on ice for 5 min
	Centrifuge 3 min at 16,000 g
	Prepare 600 µL of 95% ethanol in 1.5 mL tubes and preheat to 80°C
	Transfer 600 µL of supernatant into prepared solution above (be careful to avoid precipitate)
	Invert to mix solutions
	Centrifuge 3 min at 16,000 g
	Pipette 50 µL of solution plus 50 µL of DNA Resuspension Buffer into 1.5 mL tubes and warm in heat block
	Remove PCR tubes from freezer to thaw in centrifuge rack
	Decant alcohol from tubes and tap on absorbent paper
	Resuspend pellet in 80 µL of prewarmed DNA suspension buffer from above step
	Vortex 5 seconds
	Transfer 5 µL from sample to thawed VeriPro *Salmonella* qPCR tube
	Briefly centrifuge to settle
	Load plate and initialize GENE-UP System
	True quantification results available

Key

- Sample Prep
- Incubation
- Lysate Prep
- PCR
- Results

Quantification Workflow Comparison
MicroTally for Beef Trim

BAX® System SalQuant™	GENE-UP® Quant *Salmonella*
1 Swab	1 Swab
200 mL BAX MP	5X PBS or other media
Homogenize/stomach	Homogenize/stomach
Incubation at 42°C for 4 - 6 h	No incubation
Transfer 5 µL into lysis solution	Transfer 40 mL of sample into 50 mL tube
Heat at 37°C for 10 min	Centrifuge for 10 min into a 500 g pellet of debris
Heat at 95°C for 20 min	Transfer 25 mL of supernatant to clean tube
Cool in cold block for 5 min	Centrifuge for 10 min at 4300 g to concentrate *Salmonella*
Hydrate BAX System Real-Time PCR Assay *Salmonella* with 30 µL of lysate	Decant supernatant
Initialize and run the BAX System (75 min)	Resuspend pellet with 600 µL of Promega Nuclei Lysis Solution
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	Load plate and initialize GENE-UP System
	True quantification results available

Key

- Sample Prep
- Incubation
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