Pathogen Detection and Quantification for the Beef Industry
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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™ Level 2 Modification for Hygiena™ BAX System SalQuanUtilizing 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.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Sample Size</th>
<th>Incubation Conditions</th>
<th>Enumerable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poultry Rinsate</td>
<td>30 mL</td>
<td>42°C for 6 h</td>
<td>1 – 10,000 CFU/mL</td>
</tr>
<tr>
<td>Ground Beef</td>
<td>375 g</td>
<td>42°C for 6 h</td>
<td>1 – 10,000 CFU/g</td>
</tr>
<tr>
<td>Ground Pork</td>
<td>375 g</td>
<td>42°C for 6 h</td>
<td>1 – 10,000 CFU/g</td>
</tr>
<tr>
<td>Beef Trim</td>
<td>375 g</td>
<td>42°C for 6 h</td>
<td>1 – 10,000 CFU/g</td>
</tr>
<tr>
<td>Pork Trim</td>
<td>375 g</td>
<td>42°C for 6 h</td>
<td>1 – 10,000 CFU/g</td>
</tr>
<tr>
<td>MicroTally - Beef Trim</td>
<td>1 Swab</td>
<td>42°C for 6 h</td>
<td>1 – 10,000 CFU/mL</td>
</tr>
<tr>
<td>MicroTally - Pork Trim</td>
<td>1 Swab</td>
<td>42°C for 6 h</td>
<td>1 – 10,000 CFU/mL</td>
</tr>
</tbody>
</table>
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 MethodsSM 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 PTMSM Level 2 Modification for Hygiena BAX System SalQuant Utilizing BAX System Real-Time PCR Assay for Salmonella for Beef Matrix Extensions
**Quantification Options**

- **BAXQuant™**
  - Wide enumerable range
  - Specific CFU per sample
  - Positive CT value utilized to calculate

- **BAX System Q7**
  - Lysate Prep
  - Real-Time PCR assay
  - Run time (55-75 min.)

- **BAXLimits™**
  - Designated upper threshold
  - No specific CFU per sample
  - Positive result indicates exceeding threshold

**Limit of Quantification (LOQ)**

If SalQuant samples are negative, but positive at prevalence, the result should be \( \leq \) 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 \( < 1 \text{ CFU/mL} \)).

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

* 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 \( \geq 10 \text{ CFU/g} \)).

<table>
<thead>
<tr>
<th>Application</th>
<th>Industry</th>
<th>Segment</th>
<th>Matrix</th>
<th>Timepoint</th>
<th>Limit of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>SalQuant</td>
<td>Beef</td>
<td>Final Product</td>
<td>Ground Beef</td>
<td>5 h</td>
<td>( \geq 10 \text{ CFU/g} )</td>
</tr>
<tr>
<td>SalQuant</td>
<td>Beef</td>
<td>Final Product</td>
<td>Trim</td>
<td>4 h</td>
<td>( \geq 10 \text{ CFU/g} )</td>
</tr>
<tr>
<td>SalQuant</td>
<td>Beef</td>
<td>Final Product</td>
<td>MicroTally</td>
<td>4 h</td>
<td>( \geq 10 \text{ CFU/mL} )</td>
</tr>
</tbody>
</table>
### 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*.

1 - 10 mL D/E broth

environmental swab

Add 50 mL of pre-warmed 42°C BPW

Incubate at 42°C for 6 h (Incubate immediately after enrichment media added)

OPTIONAL

Store lysates in refrigerator for up to 24 hrs

Run samples on BAX Q7 machine utilizing the RT assay for *Salmonella* (If negative, run prevalence at 18-24 h)

Collect results from the BAX Q7 and utilize the Excel Calculator or Online Software to quantify results
## Quantification Workflow Comparison Beef Trim

### BAX® System SalQuant™
- **375 g beef trim**
- **1,500 mL BAX MP**
- Homogenize/stomach
  - Incubation at 42°C for 5 – 6 h
- Transfer 5 µL into lysis solution
- Heat at 37°C for 10 min
- Heat at 95°C for 20 min
- Cool in cold block for 5 min
- Hydrate BAX System Real-Time PCR Assay *Salmonella* with 30 µL of lysate
- Initialize and run the BAX System (75 min)
- Utilize the BAX Cycle Threshold (CT) in Excel spreadsheet or BAXQuant Online Software
- True quantification results available

### GENE-UP® Quant Salmonella
- **100 g beef trim**
- **5X PBS or other media**
- Homogenize/stomach
- No incubation
- Transfer 40 mL of sample into 50 mL tube
- Centrifuge for 10 min into a 500 g pellet of debris
- Transfer 25 mL of supernatant to clean tube
- Centrifuge for 10 min at 4300 g, to concentrate *Salmonella*
- Decant supernatant
- Resuspend pellet with 600 µL of Promega Nuclei Lysis Solution
- Vortex for 10 seconds
- 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

<table>
<thead>
<tr>
<th>BAX® System SalQuant™</th>
<th>1 Swab</th>
<th>200 mL BAX MP</th>
<th>Homogenize/stomach</th>
<th>Incubation at 42°C for 4 - 6 h</th>
<th>Transfer 5 µL into lysis solution</th>
<th>Heat at 37°C for 10 min</th>
<th>Heat at 95°C for 20 min</th>
<th>Cool in cold block for 5 min</th>
<th>Hydrate BAX System Real-Time PCR Assay Salmonella with 30 µL of lysate</th>
<th>Initialize and run the BAX System (75 min)</th>
<th>Utilize the BAX Cycle Threshold (CT) in Excel spreadsheet or BAXQuant Online Software</th>
<th>True quantification results available</th>
</tr>
</thead>
</table>

### GENE-UP® Quant Salmonella

| 1 Swab | 5X PBS or other media | Homogenize/stomach | No incubation | Transfer 40 mL of sample into 50 mL tube | Centrifuge for 10 min into a 500 g pellet of debris | Transfer 25 mL of supernatant to clean tube | Centrifuge for 10 min at 4300 g to concentrate Salmonella | Decant supernatant | Resuspend pellet with 600 µL of Promega Nuclei Lysis Solution | Vortex for 10 seconds | 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**