Pathogen Quantification Procedures Manual

INS2038-REVC

Table of Contents

Introduction

About Hygiena Quantification

The BAX System Quantification Processes, referred to as CampyQuant™, E.coliQuant™, ListeriaQuant™, SalQuant™, and VibrioQuant™ utilize shortened enrichment procedures and Real-Time PCR analysis combined with pre-designed mathematical equations to determine pre-enrichment levels of pathogens in various sample types. The BAX System is an automated molecular method for detecting microbes in food and environmental samples, combining speed and ease of use with unprecedented performance to give you fast, accurate and reliable results. The BAX System focuses on the actual genetic structure of microorganisms by detecting a unique DNA fragment found only in the target organism, simplifying PCR in your lab. All necessary reaction reagents (primers, polymerase, nucleotides and positive control) are already combined into a single tablet, conveniently packaged inside the PCR tubes you receive with each assay. This eliminates the multiple liquid transfers required in other methods and effectively reduces the potential for errors caused by the operator. The proprietary tablets also allow for efficient processing of large numbers of samples, up to 96 tests in a single batch. The automated BAX System combines PCR with fluorescent detection to significantly reduce hands-on time, minimize the potential for cross-contamination, and provide consistent results based on computerized algorithms for analysis. You simply load your prepared samples, run the program, read the results on the screen, and transfer the Cycle Threshold (CT) values into mathematical equations to compute the level of organism present prior to enrichment in original sample matrix.

About the BAX System Q7 Instrument

Your purchase of the BAX System includes the Q7 instrument with a computer workstation operating on a Microsoft Windows platform. The workstation includes removable media drives, monitor, keyboard, mouse and cables. The BAX System application is already loaded and ready for use.

Note: Although the instrument and its peripherals can be connected to a network, Hygiena cannot provide technical support for problems that arise from using this workstation on a network. Furthermore, Hygiena cannot warrant BAX System results if this computer is used with other, potentially incompatible, software.

Required Materials

Product Number	Product	Storage Conditions
KIT2039	Real-Time PCR Assay for *E. coli* O157:H7 EXACT (96 tests)	2 – 8 °C
KIT2005	Real-Time PCR Assay for *L. monocytogenes* (96 tests)	2 – 8 °C
KIT2006	Real-Time PCR Assay for *Salmonella* (96 tests)	2 – 8 °C
KIT2010	Real-Time PCR Assay for *Vibrio* (96 tests	2 – 8 °C
KIT2018	Real-Time PCR Assay for *Campylobacter jejuni/coli/lari* (96 tests)	2 – 8 °C
KIT2019	Real-Time PCR Assay for Genus *Listeria* (96 tests)	2 – 8 °C
MED2010	Buffered Peptone Water (2.5 kg)	Room Temperature 2 – 30 °C
MED2003 MED2016 MED2029	BAX System MP Media (2.5 kg, 10 kg, STAT packs)	Room Temperature 10 – 25 °C
MED2032	Quant™ Solution (25 mL)	2 – 8 °C
ASY2018 ASY2020	BAX System Q7 Start-Up Package (equipment and supplies for 192 initial tests)	Specifications available at **www.hygiena.com**

Quant Solution Product Description

- Storage conditions: 2 – 8 °C
- Appearance: colorless/clear
- Shelf-Life: 2 years under proper storage conditions from manufacturing date
- Volume: 25 mL bottle

Directions

1. Prepare 1 L of BAX MP media to manufacturer's specifications
2. Cool to 45 – 55 °C
3. Aseptically transfer 0.5 or 1.0 mL of Quant Solution, depending on the protocol, into 1 L of cooled BAX MP media and mix

Materials Handling, Storage and Disposal

Cycler/Detector

The instrument requires a constant supply of air that is 31 °C or cooler in order 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 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 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

Reagents should be used by the expiration date stamped on the individual labels.

Reagent packages should be kept refrigerated at 2-8 °C. Do not freeze. If storing PCR tubes with tablets in an open kit for more than 3 weeks 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.*

After protease has been added to the lysis buffer, shelf life of the solution is two weeks when stored at 2-8 °C.

Cooling blocks should be kept refrigerated at 2-8 °C and used within 30 minutes of removal from refrigerator.

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.

Limit of Quantification (LOQ)

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

Application	Industry	Segment	Matrix	Timepoint	Limit of Quantification
SalQuant	Poultry	Primary Production	Feed	8 h	10 CFU/g
SalQuant	Poultry	Primary Production	Boot Swabs	10 h	10 CFU/mL
SalQuant	Poultry	Primary Production	Dust Swabs	12 h	100 CFU/mL
SalQuant	Poultry	Primary Production	Cecal Tonsils	10 h	10 CFU/mL
SalQuant	Poultry	Primary Production	Feet Swabs	10 h	10 CFU/mL
SalQuant	Poultry	Primary Production	Cloacal Swabs	10 h	10 CFU/mL
SalQuant	Poultry	Primary Production	Poult Pads (25 g)	8 h	1 CFU/g
SalQuant	Poultry	Processing	Crop	6 h	10 CFU/g
SalQuant	Poultry	Processing	Lungs	6 h	10 CFU/g
SalQuant	Poultry	Processing	Spleens	6 h	1 CFU/mL
SalQuant	Poultry	Processing	Livers	6 h	1 CFU/mL
SalQuant	Poultry	Processing	Carcass Swabs	6 h	1 CFU/mL
SalQuant	Poultry	Processing	Rinsate	6 h	1 CFU/mL
SalQuant	Poultry	Processing	Rinsate (low level)	10 h	0.5 CFU/30 mL Sample
CampyQuant	Poultry	Processing	Rinsate	20 h	10 CFU/mL
SalQuant	Poultry	Final Product	Ground Turkey (1:1)	8 h	1 CFU/g
SalQuant	Poultry	Final Product	Ground Turkey (1:4)	8 h	1 CFU/g
SalQuant	Poultry	Final Product	Ground Turkey (1:6)	8 h	1 CFU/g
SalQuant	Poultry	Final Product	Ground Turkey	0 h	100,000 CFU/g
SalQuant	Poultry	Final Product	Ground Chicken (1:1)	8 h	1 CFU/g
SalQuant	Poultry	Final Product	Ground Chicken (1:4)	8 h	1 CFU/g
SalQuant	Poultry	Final Product	Ground Chicken (1:6)	8 h	1 CFU/g
SalQuant	Beef	Primary Production	Boot Swab	6 h	10 CFU/mL
SalQuant	Beef	Primary Production	MicroTally Drain Swab	6 h	1 CFU/mL
SalQuant	Beef	Primary Production	Feces	8 h	10 CFU/g
SalQuant	Beef	Primary Production	Feces (high level)	0 h	100,000 CFU/g
SalQuant	Beef	Processing	Lymph Nodes	6 h	10 CFU/Lymph Node
SalQuant	Beef	Processing	Cecal Swab	8 h	10 CFU/mL
SalQuant	Beef	Processing	Cecal Contents	8 h	1 CFU/g
SalQuant	Beef	Processing	Cecal Contents (high level)	0 h	100,000 CFU/g
SalQuant	Beef	Processing	Carcass Swab	8 h	10 CFU/Swab
E.coliQuant	Beef	Processing	Carcass Swab	8 h	10 CFU/Swab
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

Limit of Quantification (LOQ), continued

Application	Industry	Segment	Matrix	Timepoint	Limit of Quantification
SalQuant	Pork	Primary Production	Boot Swab	8 h	1 CFU/mL
SalQuant	Pork	Primary Production	Feces	10 h	1 CFU/g
SalQuant	Pork	Primary Production	Feces (high level)	0 h	100,000 CFU/g
SalQuant	Pork	Processing	Head Trim Rinsate	8 h	1 CFU/mL
SalQuant	Pork	Processing	Carcass Swab	6 h	1 CFU/mL
SalQuant	Pork	Processing	Lymph Nodes (small)	6 h	10 CFU/Lymph Node
SalQuant	Pork	Processing	Lymph Nodes (medium)	6 h	10 CFU/Lymph Node
SalQuant	Pork	Processing	Ground Pork	7 h	1 CFU/g
SalQuant	Pork	Final Product	Pork Trim	6 h	1 CFU/g
SalQuant	Pork	Final Product	MicroTally	6 h	1 CFU/mL
VibrioQuant	Oysters	Final Product	Oysters	8 h	1,000 CFU/mL
ListeriaQuant	Environmental	Environmental	Swabs	16 h	1 CFU/Swab
SalQuant	Environmental	Environmental	Swabs	6 h	1 CFU/mL
SalQuant	Laboratory	Laboratory	Pure	0 h	1,000 CFU/mL
ListeriaQuant	Laboratory	Laboratory	Pure	0 h	1,000 CFU/mL
VibrioQuant	Laboratory	Laboratory	Pure	0 h	1,000 CFU/mL
E.coliQuant	Laboratory	Laboratory	Pure	0 h	1,000 CFU/mL

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). See table below for specific matrix and application.

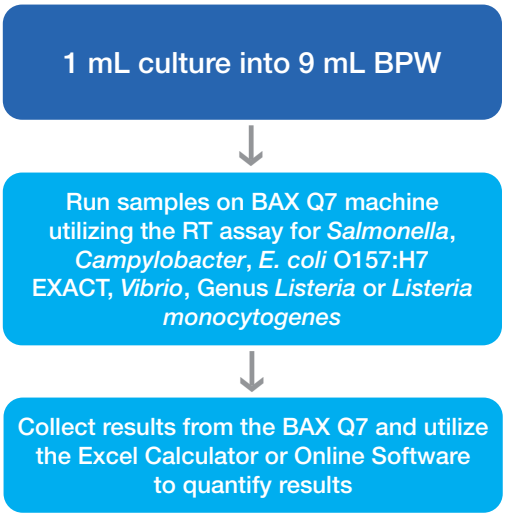
Application	Industry	Segment	Matrix	Tiimepoint	Limit of Detection
SalQuant	Poultry	Final Product	Ground Beef	4 h	≥ 10 CFU/mL
SalQuant	Beef	Final Product	Rinsate	5 h	≥ 10 CFU/g
SalQuant	Beef	Final Product	Trim	4 h	≥ 10 CFU/g
SalQuant	Beef	Final Product	MicroTally	4 h	≥ 10 CFU/mL
SalQuant	Pork	Final Product	Ground Pork	5 h	≥ 10 CFU/g
SalQuant	Pork	Final Product	Pork Trim	4 h	≥ 10 CFU/g
SalQuant	Pork	Final Product	MicroTally	4 h	≥ 10 CFU/g
SalQuant	Pork	Processing	Carcass Swab	4 h	≥ 10 CFU/mL
VibrioQuant	Oysters	Final Product	Oysters	6 h	≥ 10 CFU/g

Pure Culture

Dilution & PCR Procedure

Create dilutions (1 mL culture into 9 mL BPW) of the overnight culture from 10^{-1} to 10^{-4} of *Salmonella, Campylobacter, E. coli, Vibrio*, Genus *Listeria*, or *Listeria monocytogenes*. Choose from any dilution created (10^{-1} to 10^{-4}) and transfer 5 µL into cluster tubes containing lysis buffer to start the PCR process.

Perform PCR analysis with the BAX System Real-Time PCR Assays for *Salmonella*, *Campylobacter, E. coli* O157:H7 EXACT, *Vibrio*, Genus *Listeria*, or *Listeria monocytogenes.* Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results. View more instructions for calculators on pages 46-50.



Beef
Primary Production

Boot Swabs - *Salmonella*

Enrichment & PCR Procedure

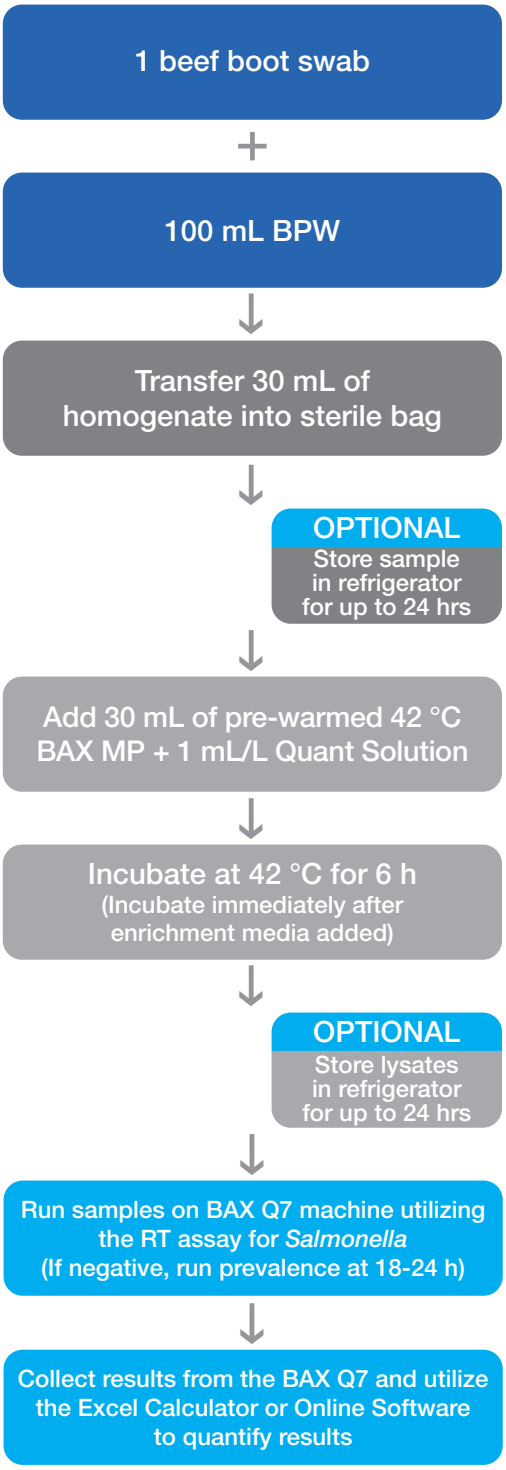
Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution.

Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 6 h.

Following 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. View more instructions for calculators on pages 46-50.

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
Primary Production

Feces- *Salmonella*

Enrichment & PCR Procedure

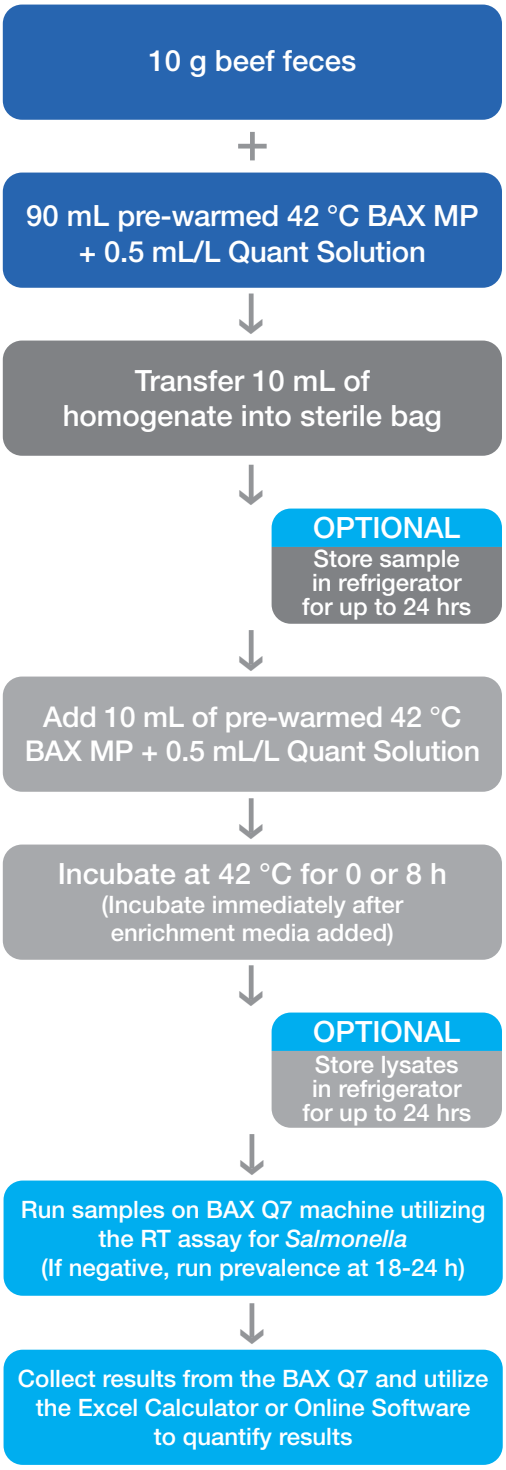
Add 10 g of beef feces to 90 mL of pre-warmed 42 °C BAX MP + 0.5 mL/L Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds.

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.

Incubate sample at 42 ± 1 °C for 0 h for 100,000 – 100,000,000 CFU/g enumerable range or 8 h for 10 – 10,000 CFU/g enumerable range.

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. View more instructions for calculators on pages 46-50.

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
Primary Production

MicroTally Feedlot Drain Swabs - *Salmonella*

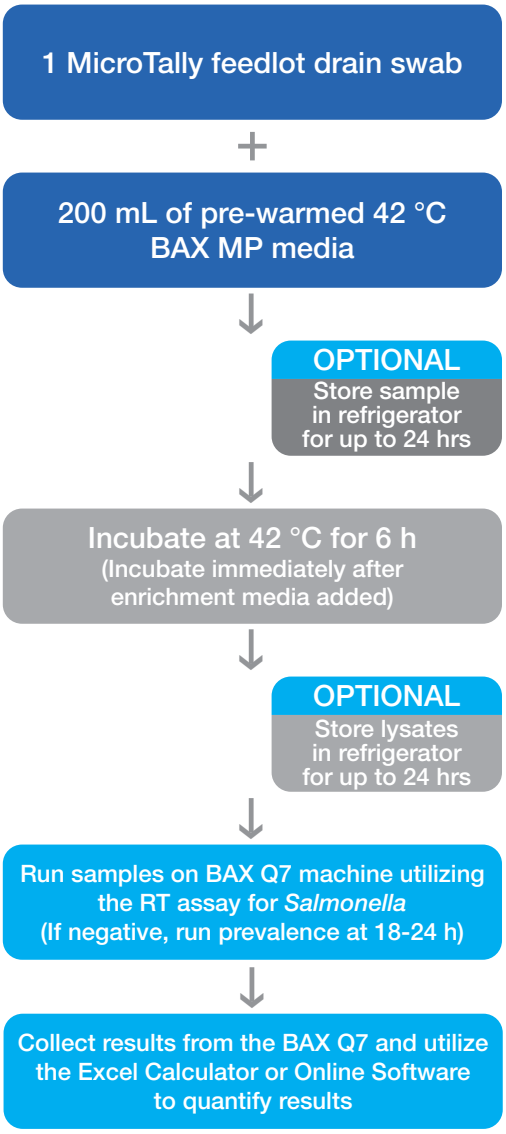
Enrichment & PCR Procedure

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 6 h for 1 – 10,000 CFU/mL 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. View more instructions for calculators on pages 46-50.

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

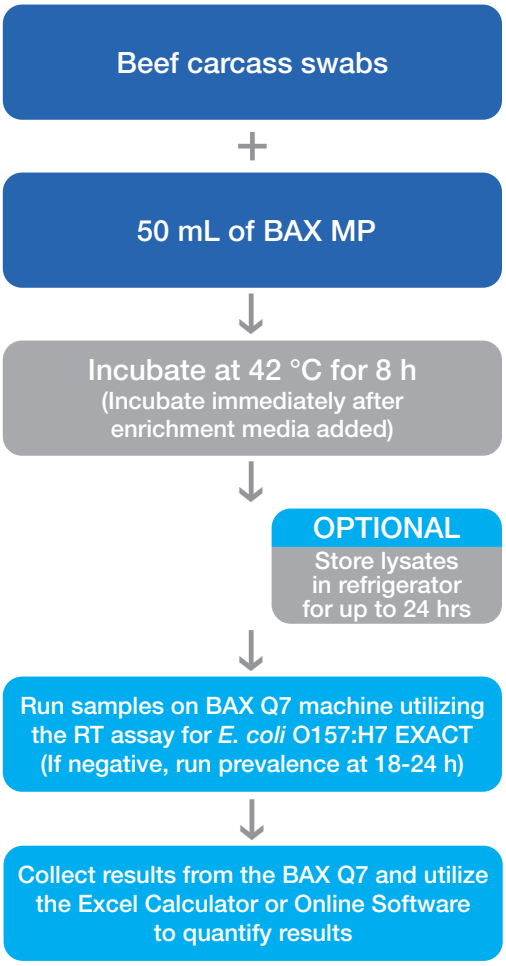
Carcass Swabs - *E. coli* O157:H7 Enrichment & PCR Procedure

Swab a beef carcass with a BPW pre-moistened swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1 °C for 8 h.

Following enrichment for quantification, perform PCR analysis with the BAX System Real-Time PCR Assay for *E. coli* O157:H7 EXACT. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for the remainder of the 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *E. coli* O157:H7 EXACT.



Beef Processing

Carcass Swabs - *Salmonella*

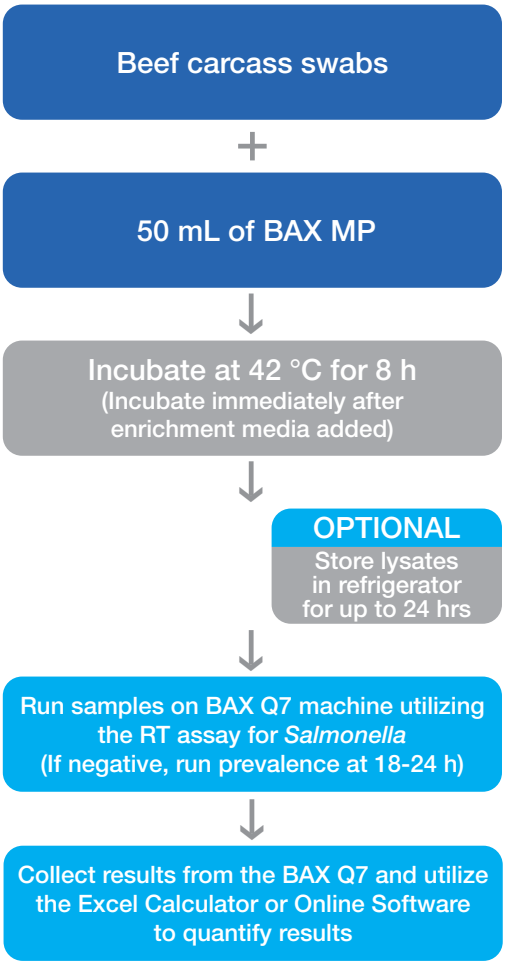
Enrichment & PCR Procedure

Swab a beef carcass with a BPW pre-moistened swab and combine with 50 mL of pre-warmed (42 °C) BAX MP media as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1 °C for 8 h.

Following 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. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for the remainder of the 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef Processing

Cecal Contents - *Salmonella*

Enrichment & PCR Procedure

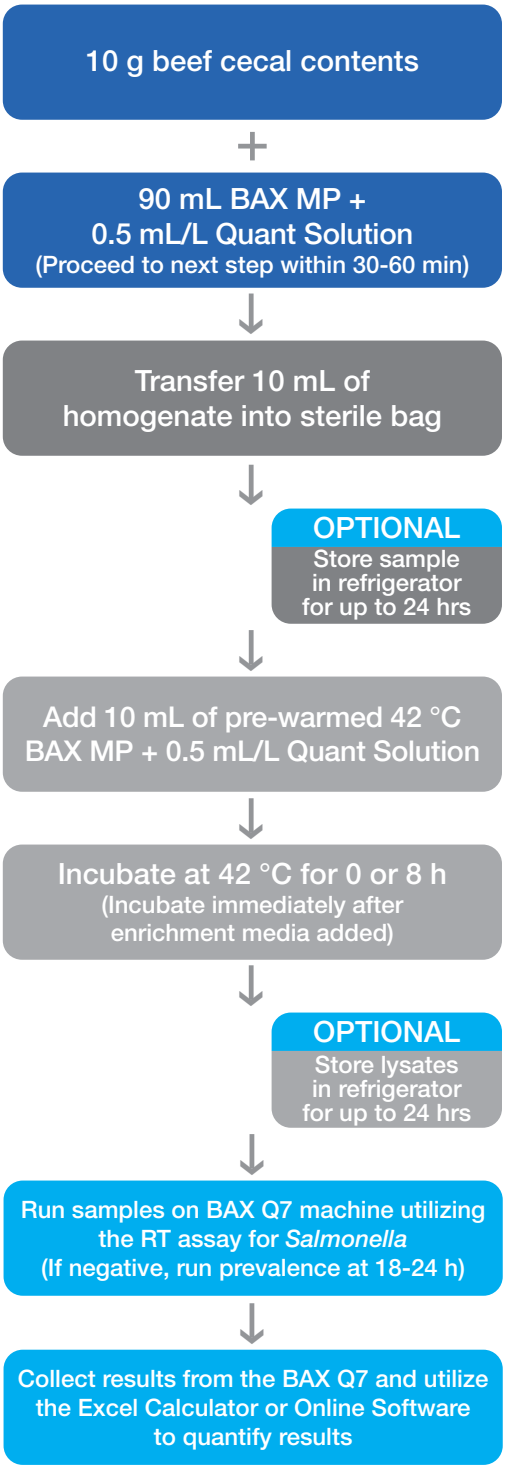
Add 10 g of beef cecal contents to 90 mL of BAX MP with + 0.5 mL/L Quant Solution as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

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.

Incubate sample at 42 ± 1 °C for 0 h for 100,000 – 100,000,000 CFU/g enumerable range or 8 h for 1 – 1,000 CFU/g enumerable range.

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. View more instructions for calculators on pages 46-50.

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

Cecal Swabs - *Salmonella*

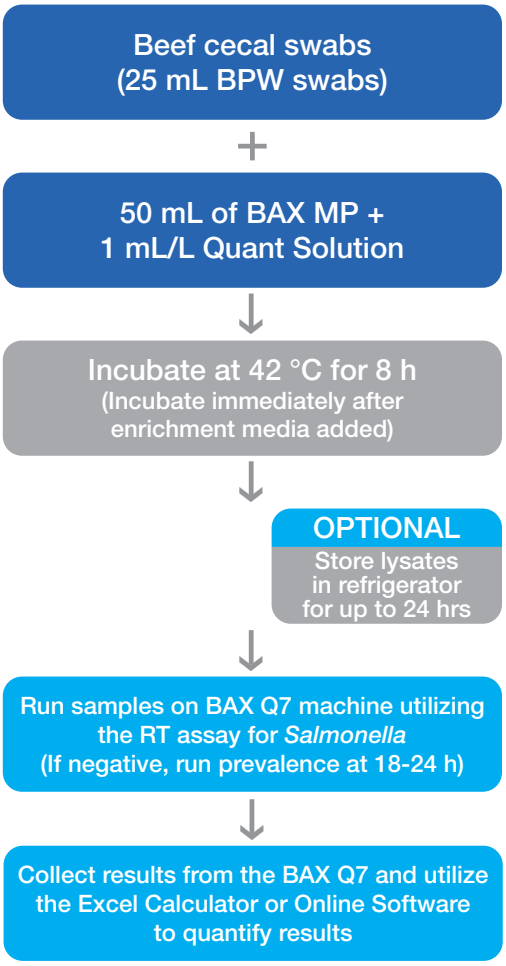
Enrichment & PCR Procedure

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. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for the remainder of the 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Beef Processing

Lymph Nodes - *Salmonella*

Enrichment & PCR Procedure

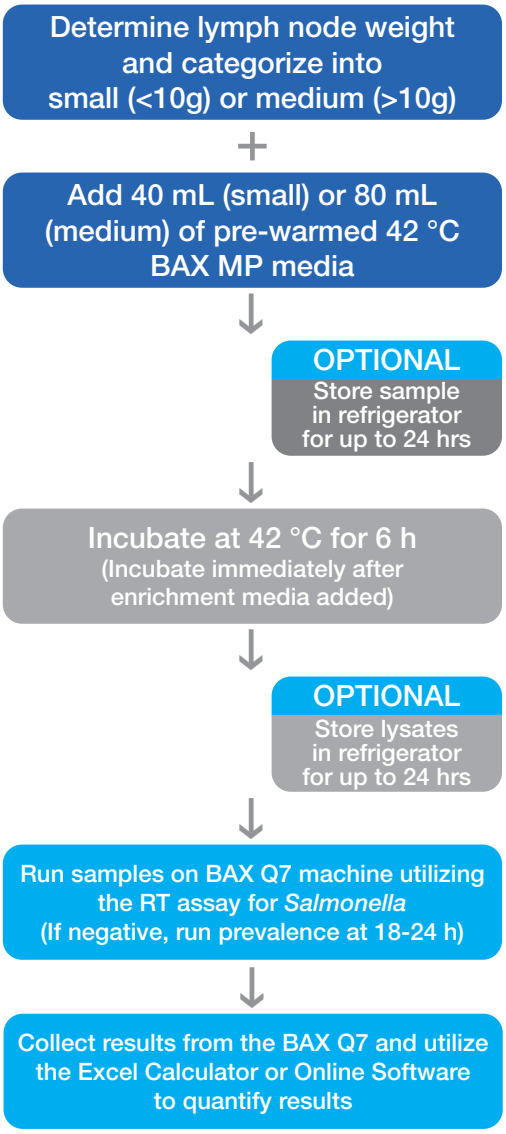
Weigh and process lymph nodes into small (<10 g) or medium (>10 g) size category.

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. View more instructions for calculators on pages 46-50.

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 - *Salmonella*

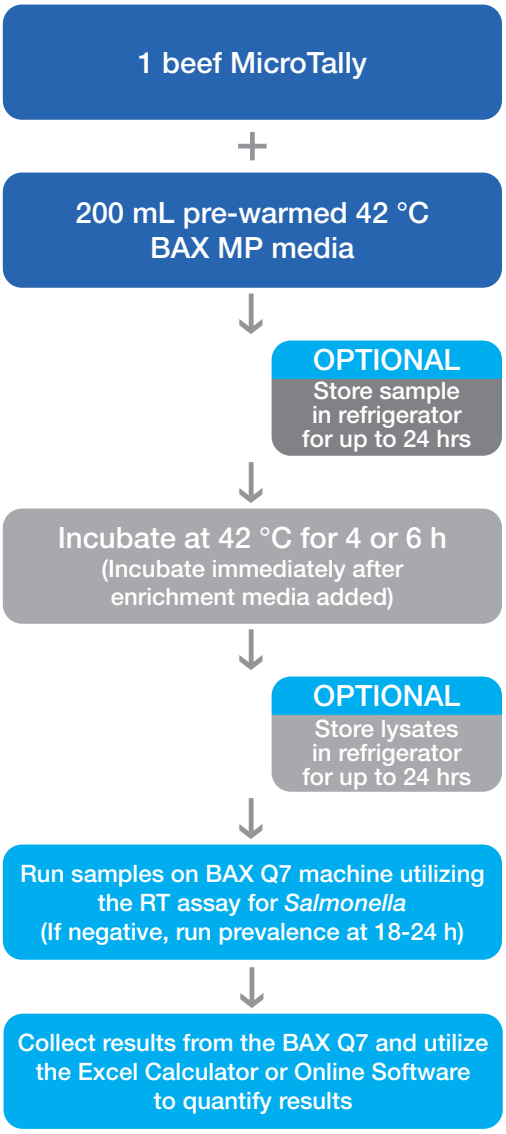
Enrichment & PCR Procedure

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. View more instructions for calculators on pages 46-50.

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

Trim - *Salmonella*

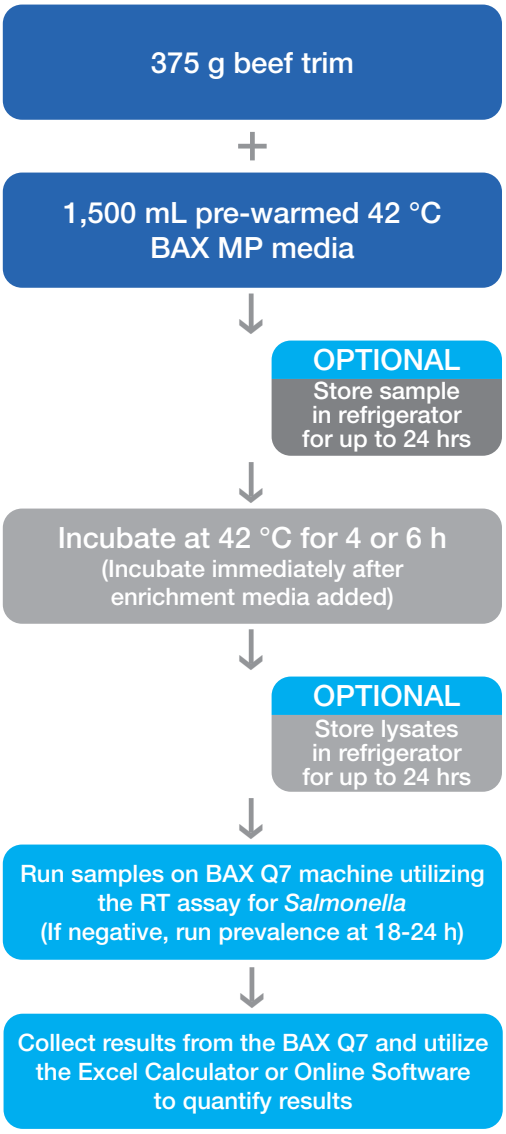
Enrichment & PCR Procedure

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. View more instructions for calculators on pages 46-50.

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 - *Salmonella*

Enrichment & PCR Procedure

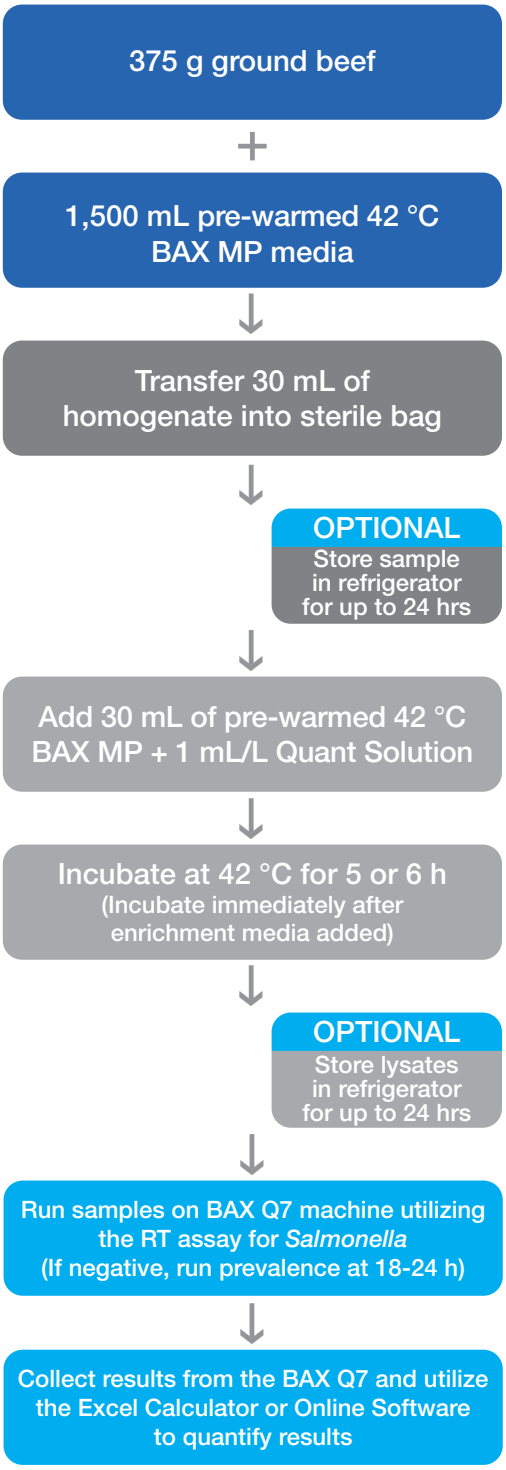
Add 375 g of ground beef to 1,500 mL of pre-warmed (42 °C) BAX® MP media as Primary Enrichment. Homogenize at 230 RPM for 30 seconds

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. View more instructions for calculators on pages 46-50.

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 - Genus *Listeria* and *Listeria monocytogenes*

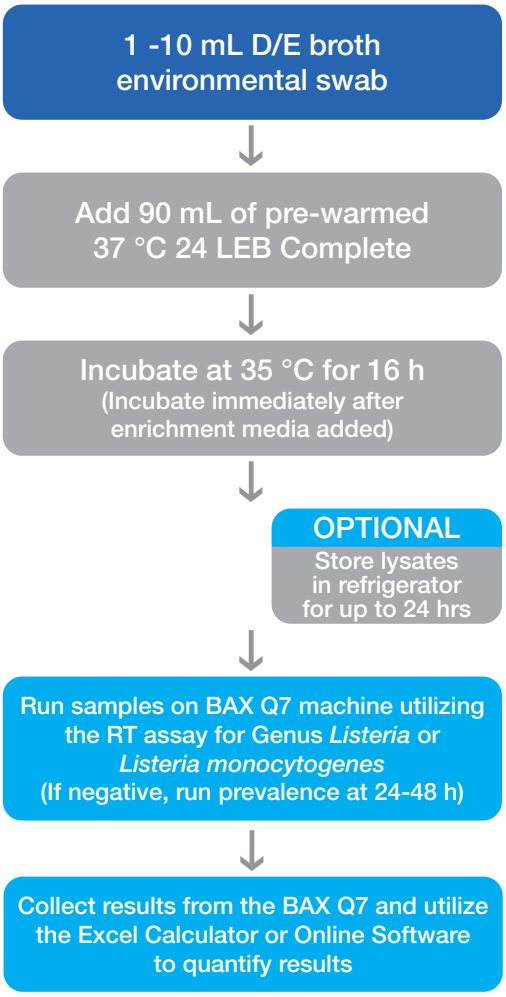
Enrichment & PCR Procedure

Add 1 environmental swab to 90 mL of pre-warmed (37 °C) 24 LEB Complete media as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 35 ± 1 °C for 16 h.

Following enrichment for quantification, perform PCR analysis with the BAX System Real-Time PCR Assay for Genus *Listeria* or *Listeria monocytogenes*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for the remainder of the 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for Genus *Listeria* or *Listeria monocytogenes*.



Environmental Monitoring

Swab - D/E Broth - *Salmonella*

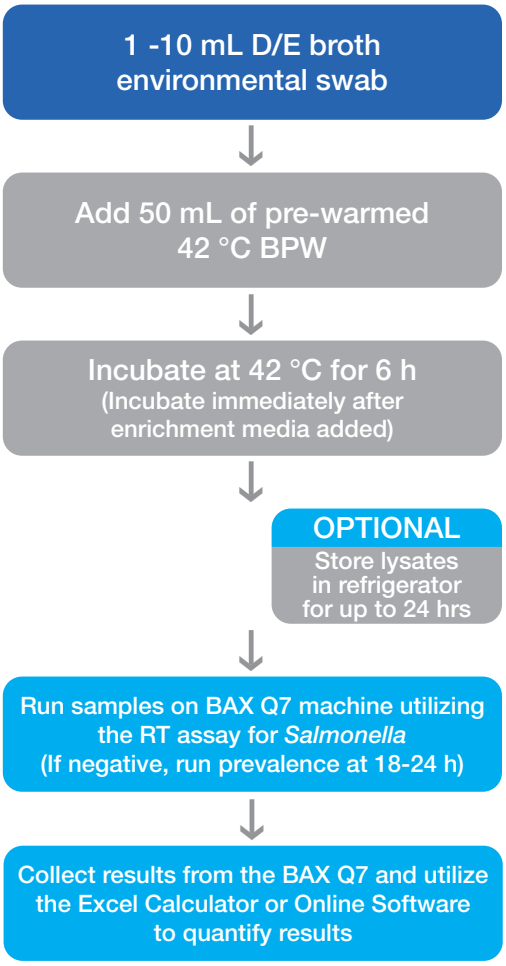
Enrichment & PCR Procedure

Add 1 D/E broth environmental swab to BPW media for the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1 °C for 6 h.

Following 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. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for the remainder of the 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Pork
Primary Production

Boot Swabs - *Salmonella*

Enrichment & PCR Procedure

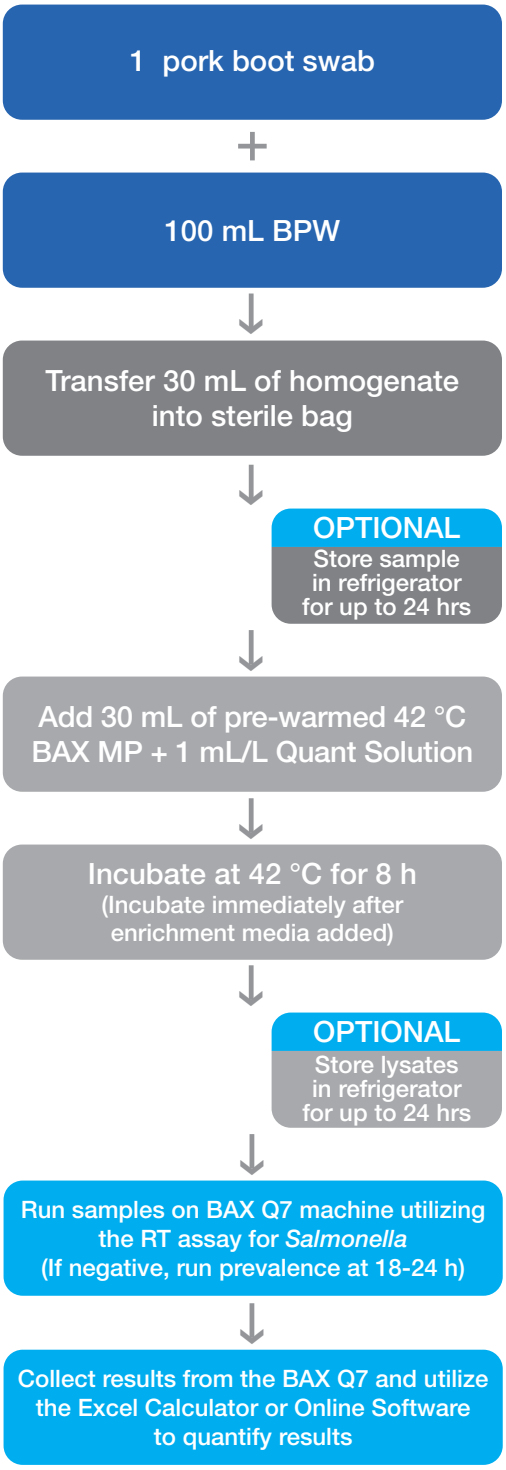
Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution.

Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 8 h.

Following 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. View more instructions for calculators on pages 46-50.

After transferring the 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*.



Pork
Primary Production

Feces - *Salmonella*

Enrichment & PCR Procedure

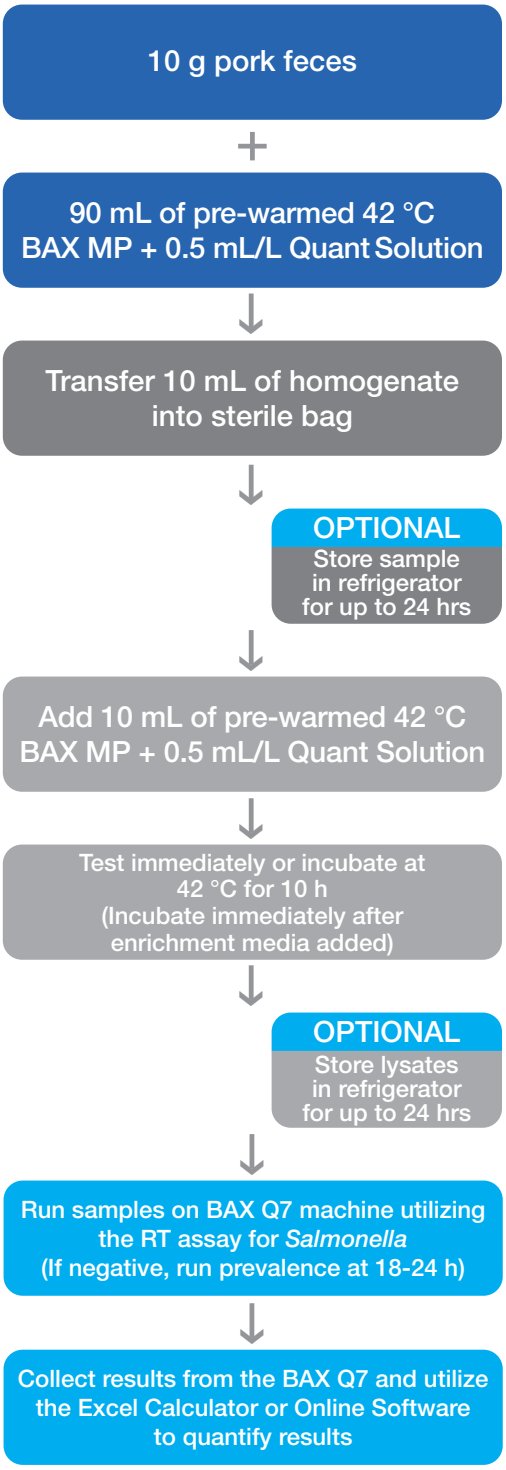
Add 10 g of pork feces to 90 mL of pre-warmed (42 °C) BAX MP + 0.5 mL/L of BAX Quant Solution as Primary Enrichment. Homogenize by hand for 60 seconds.

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

Test immediately for 100,000 - 100,000,000 CFU/g enumerable range, or incubate sample at 42 ± 1 °C for 10 h for 1 - 10,000 CFU/g enumerable range.

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. View more instructions for calculators on pages 46-50.

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*.



Pork Processing

Carcass Swab - *Salmonella*

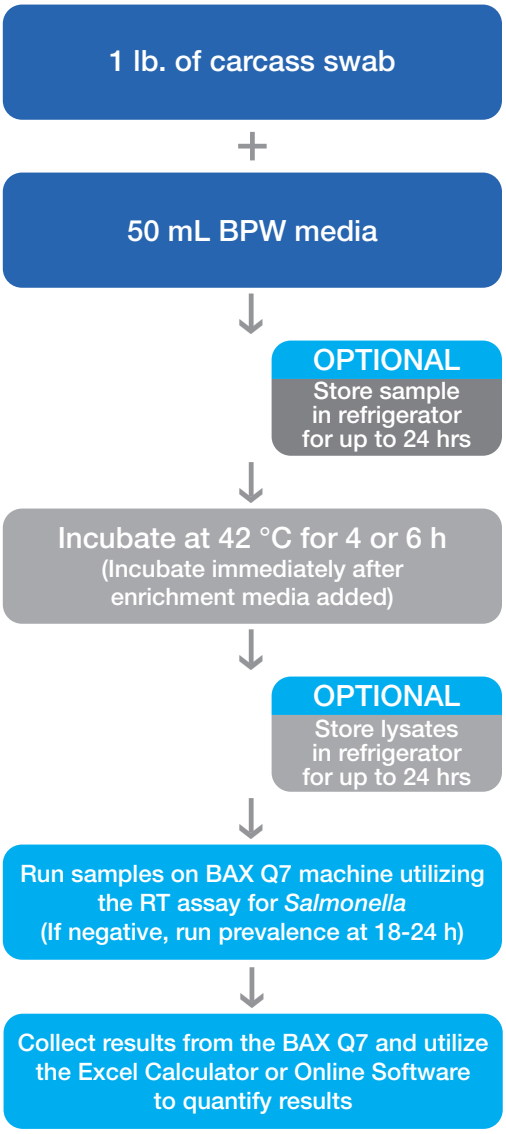
Enrichment & PCR Procedure

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 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. View more instructions for calculators on pages 46-50.

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*.



Pork Processing

Head Trim Rinse - *Salmonella* Enrichment & PCR Procedure

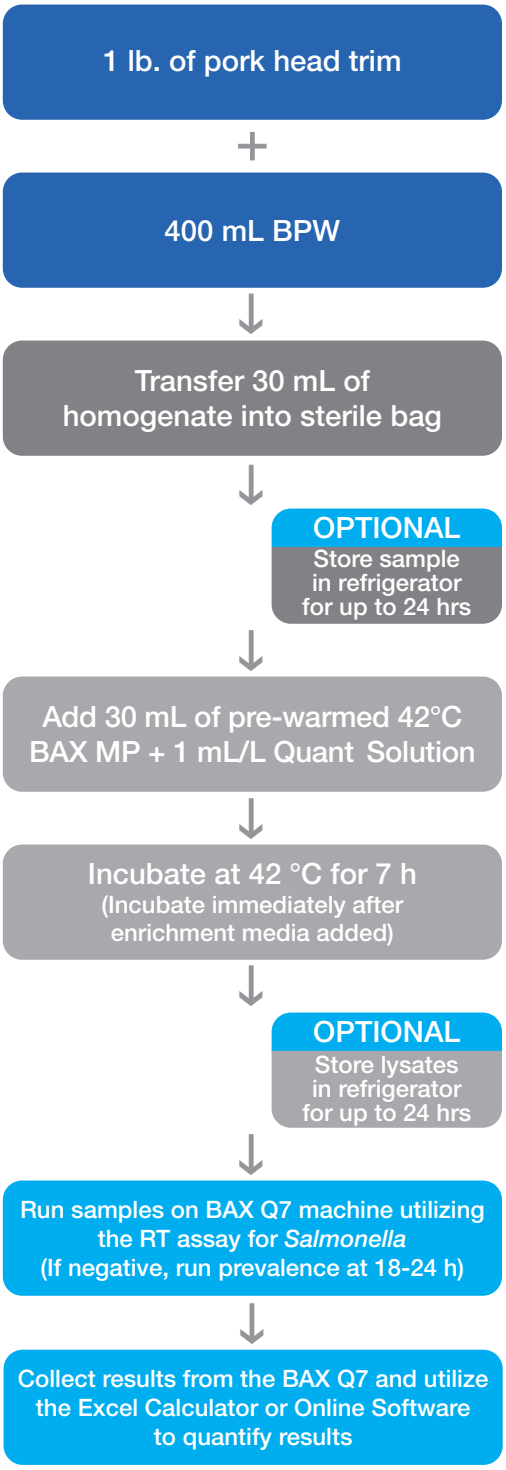
Add 1 lb. of head trim to 400 mL of BPW as Primary Enrichment. Homogenize by hand for 60 seconds.

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

Incubate sample at 42 ± 1 °C for 7 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Pork Processing

Lymph Nodes - *Salmonella*

Enrichment & PCR Procedure

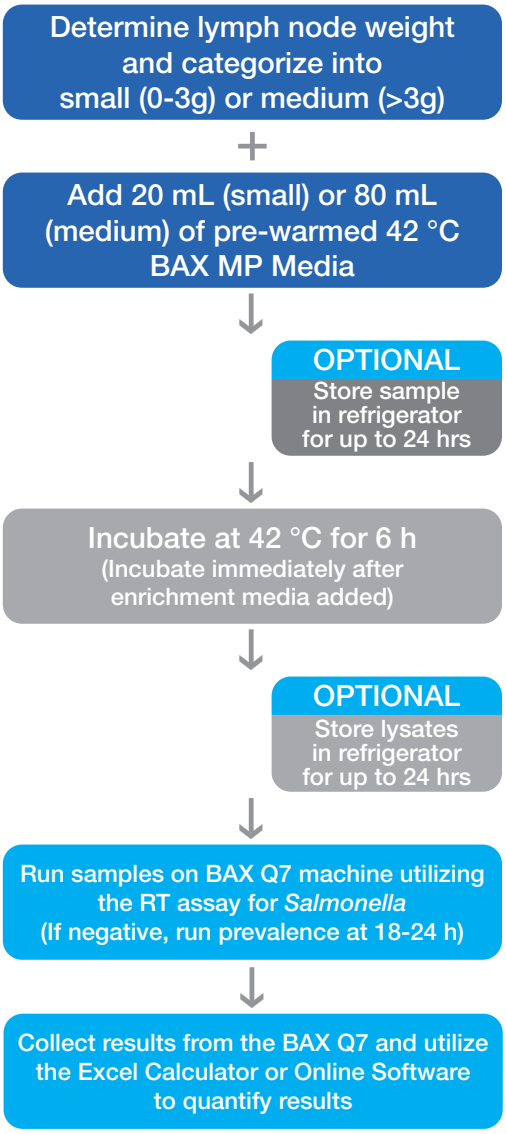
Weigh and process lymph nodes into small (0 - 3 g) or medium (3.1 - 25 g) size category.

For small nodes, add 20 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. View more instructions for calculators on pages 46-50.

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*.



Pork
Final Product

Ground Pork - *Salmonella*

Enrichment & PCR Procedure

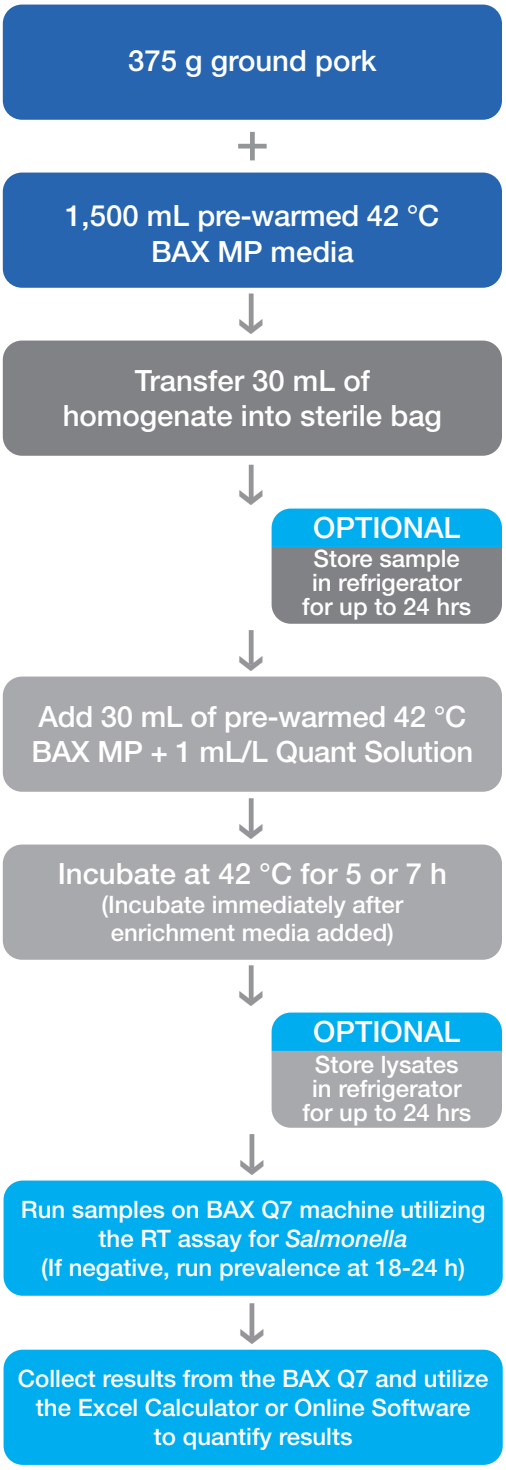
Add 375 g of ground pork to 1,500 mL of pre-warmed (42 °C) BAX MP media as Primary Enrichment. Homogenize at 230 RPM for 30 seconds

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 7 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. View more instructions for calculators on pages 46-50.

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*.



Pork
Final Product

MicroTally - *Salmonella*

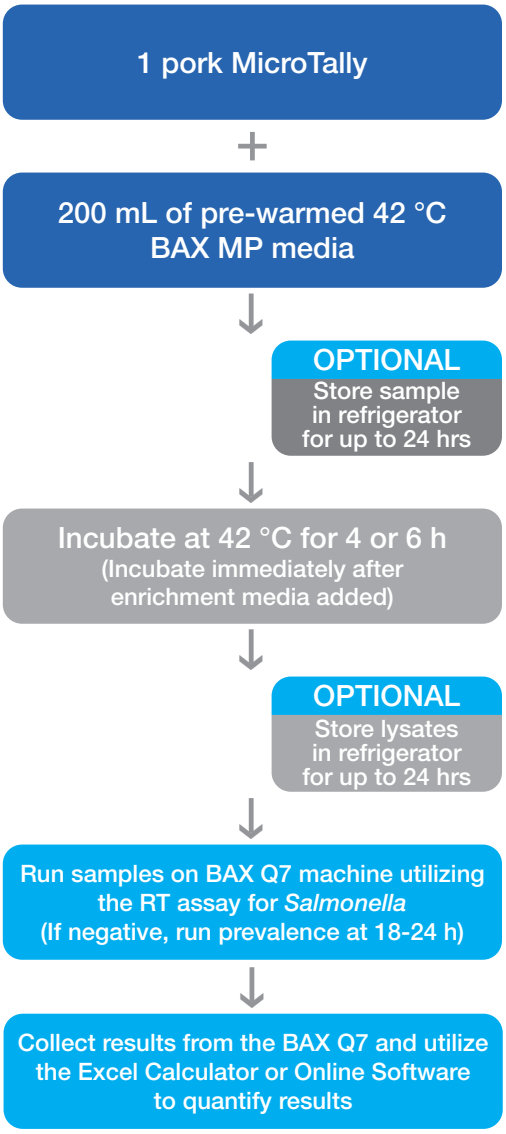
Enrichment & PCR Procedure

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. View more instructions for calculators on pages 46-50.

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*.



Pork
Final Product

Trim - *Salmonella*

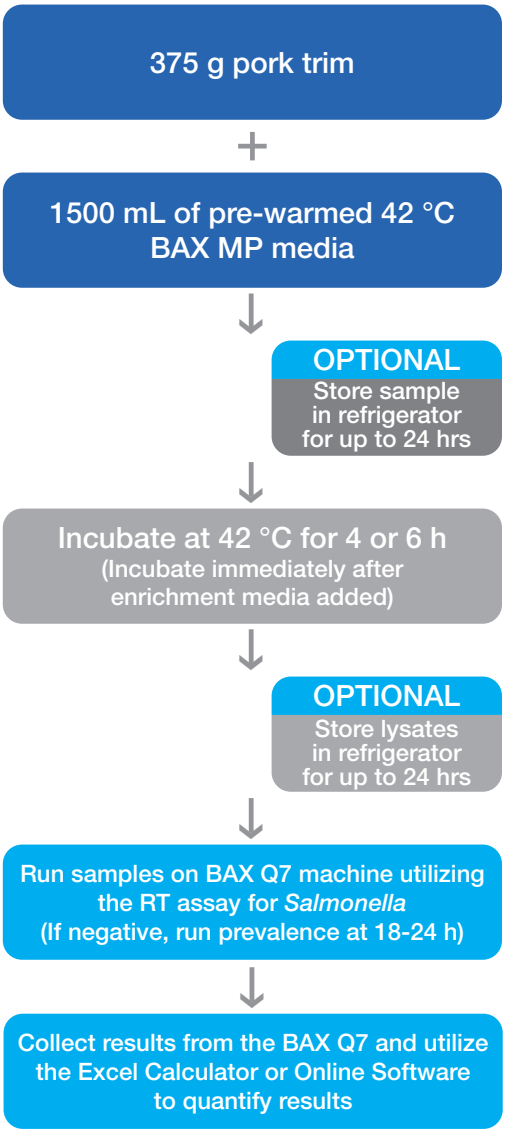
Enrichment & PCR Procedure

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

Incubate sample at 42 ± 1 °C for 4 h for LOD10 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Boot Swabs - *Salmonella*

Enrichment & PCR Procedure

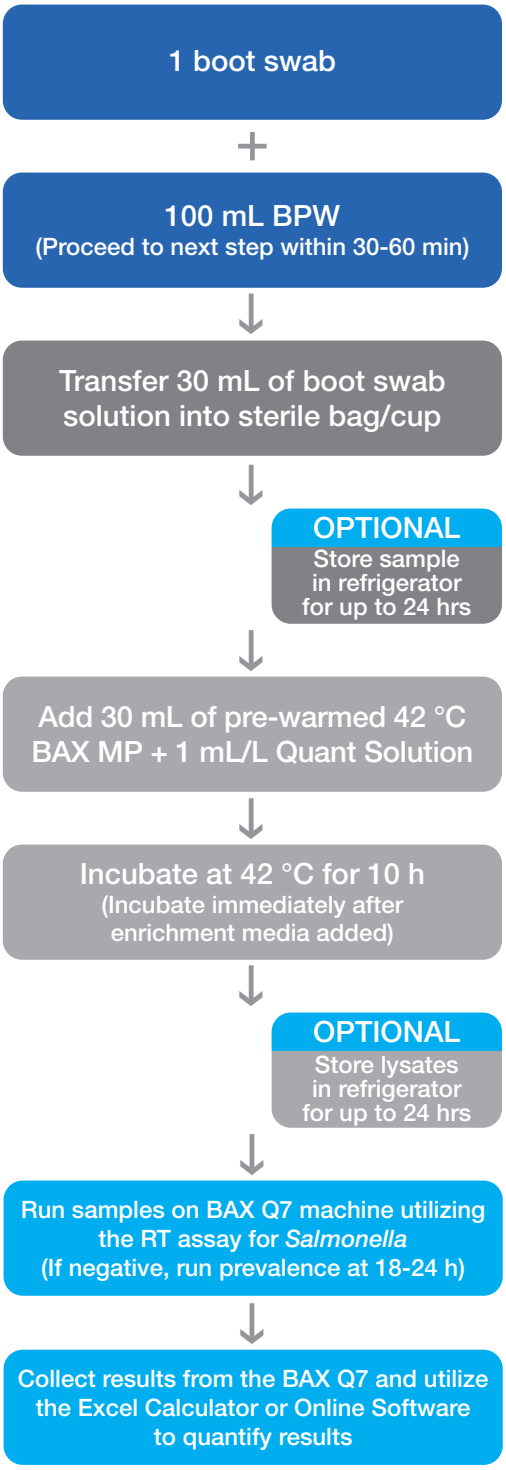
Add 1 boot swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution.

Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h.

Following 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. View more instructions for calculators on pages 46-50.

After transferring the 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*.



Poultry
Primary Production

Cecal Tonsils - *Salmonella*

Enrichment & PCR Procedure

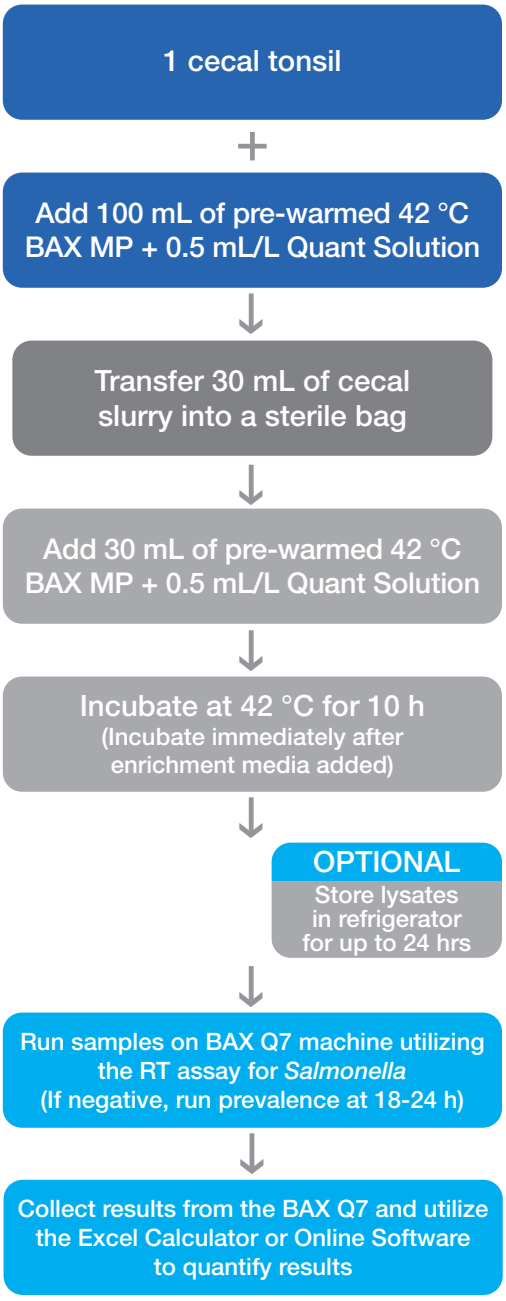
Add 1 cecal tonsil to pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

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

Incubate sample at 42 ± 1 °C for 10 h.

Following 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. View more instructions for calculators on pages 46-50.

After transferring an 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*.



Poultry
Primary Production

Cloacal Swabs - *Salmonella*

Enrichment & PCR Procedure

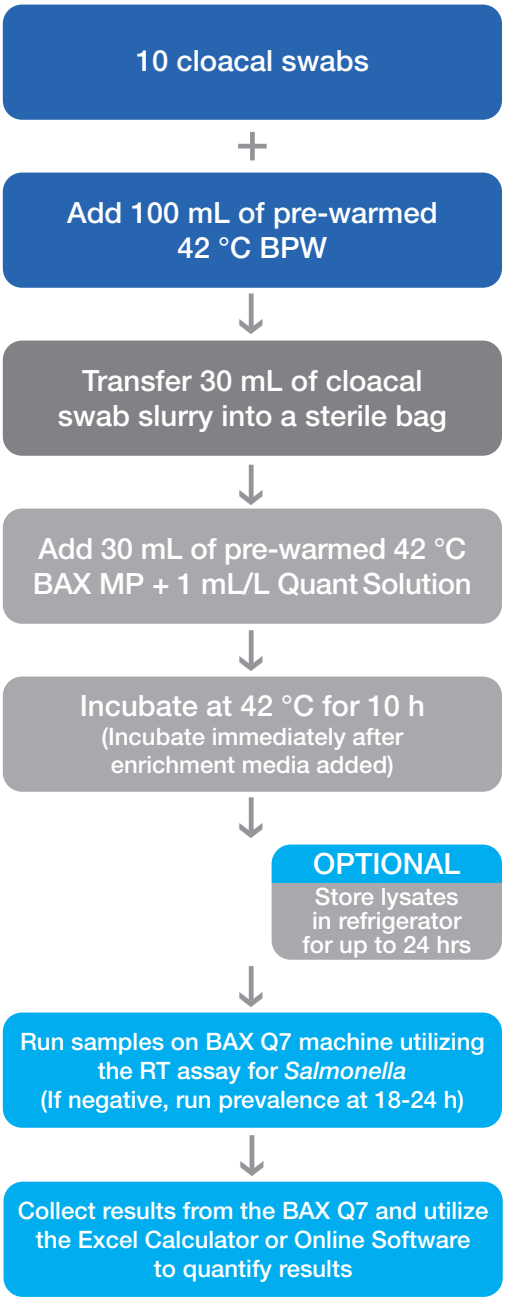
Add a composite of 10 cloacal swabs to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution.

Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Crop - *Salmonella*

Enrichment & PCR Procedure

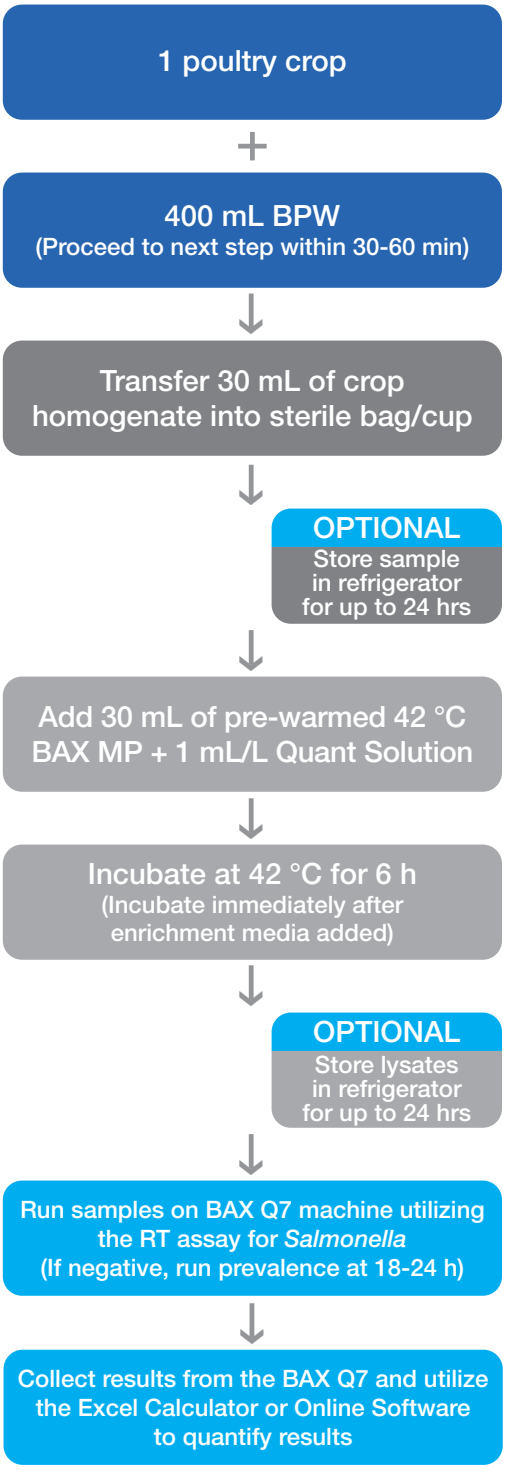
Add 1 poultry crop to 400 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

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

Incubate sample at 42 ± 1 °C for 6 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Dust Swabs - *Salmonella*

Enrichment & PCR Procedure

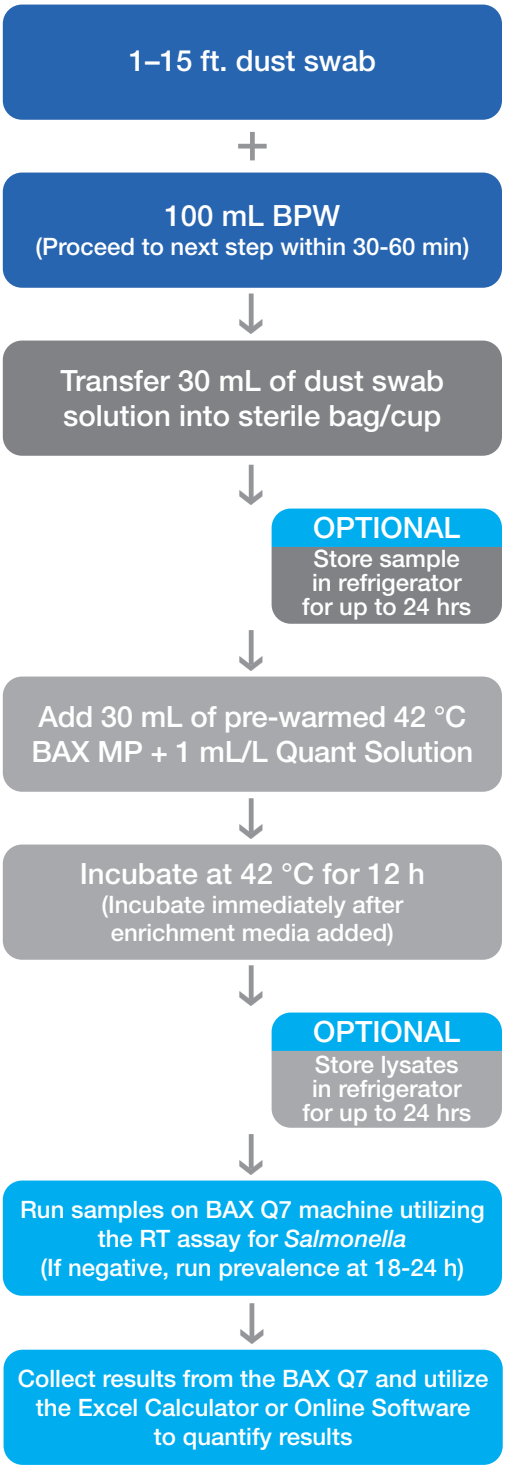
Add 1 dust swab to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution.

Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 12 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Feed - *Salmonella*

Enrichment & PCR Procedure

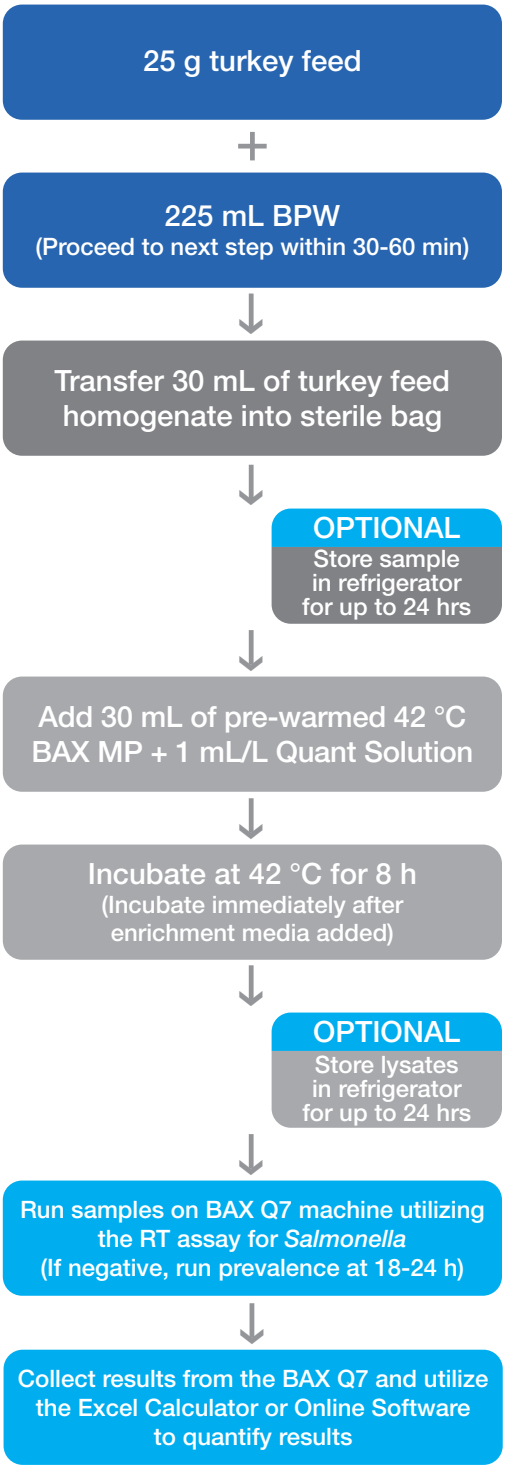
Add 25 g of poultry (turkey) feed to 225 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

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

Incubate sample at 42 ± 1 °C for 8 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Feet Swabs - *Salmonella*

Enrichment & PCR Procedure

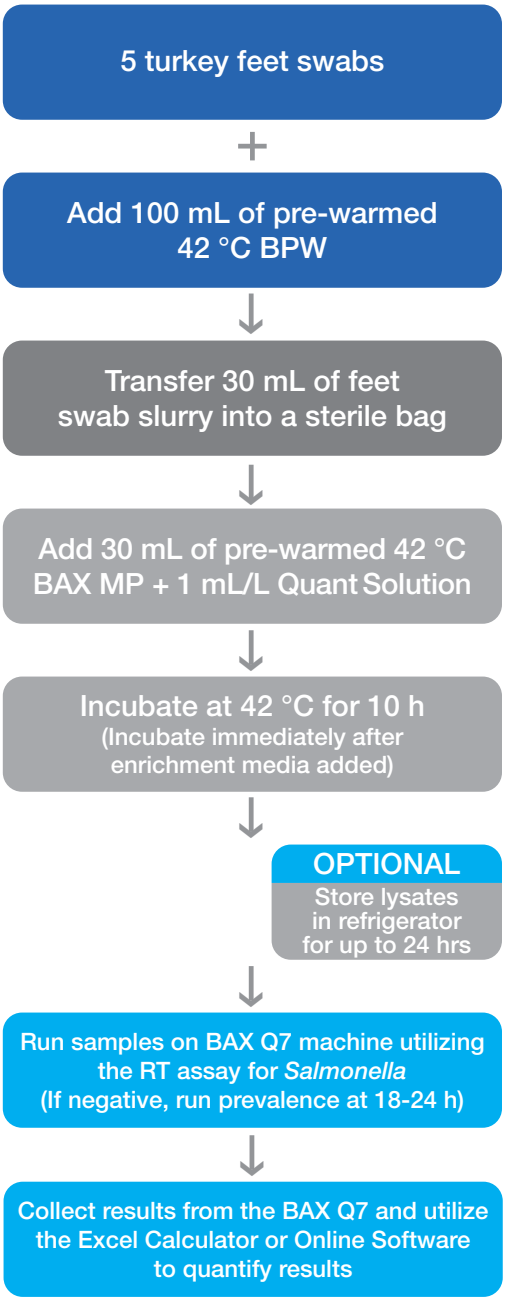
Add a composite of 5 turkey feet swabs to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Transfer 30 mL of Primary Enrichment into a sterile container with 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution.

Hand massage for 15-30 seconds for homogenization. Incubate sample at 42 ± 1 °C for 10 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Liver - *Salmonella*

Enrichment & PCR Procedure

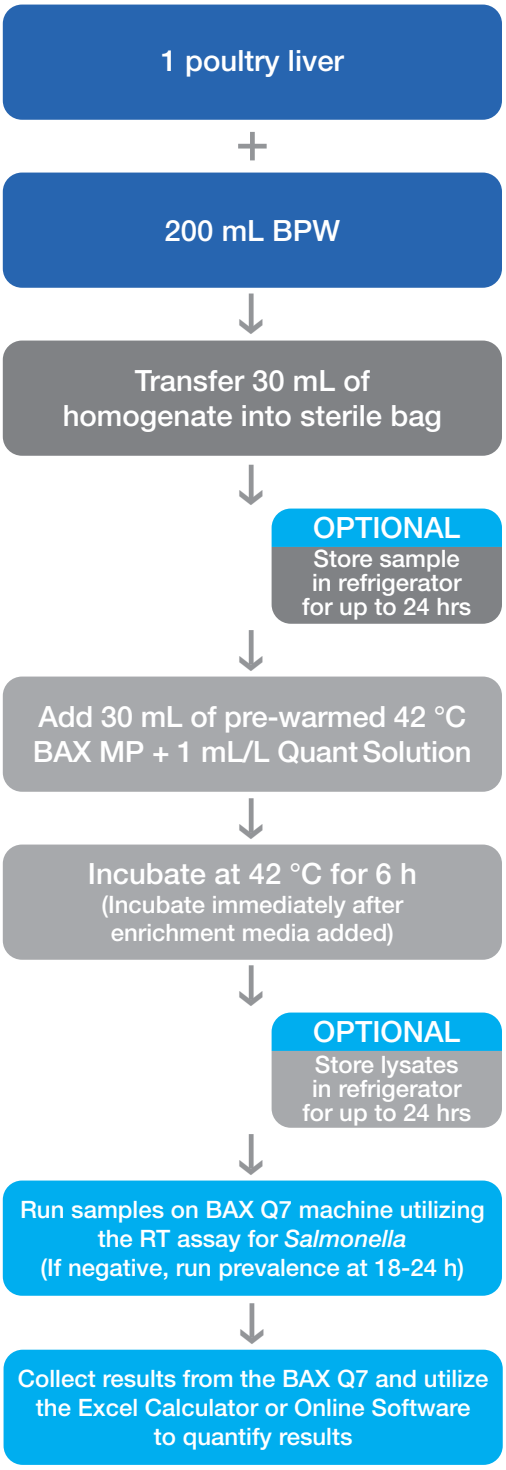
Add 1 poultry liver to 200 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

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

Incubate sample at 42 ± 1 °C for 6 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Lungs - *Salmonella*

Enrichment & PCR Procedure

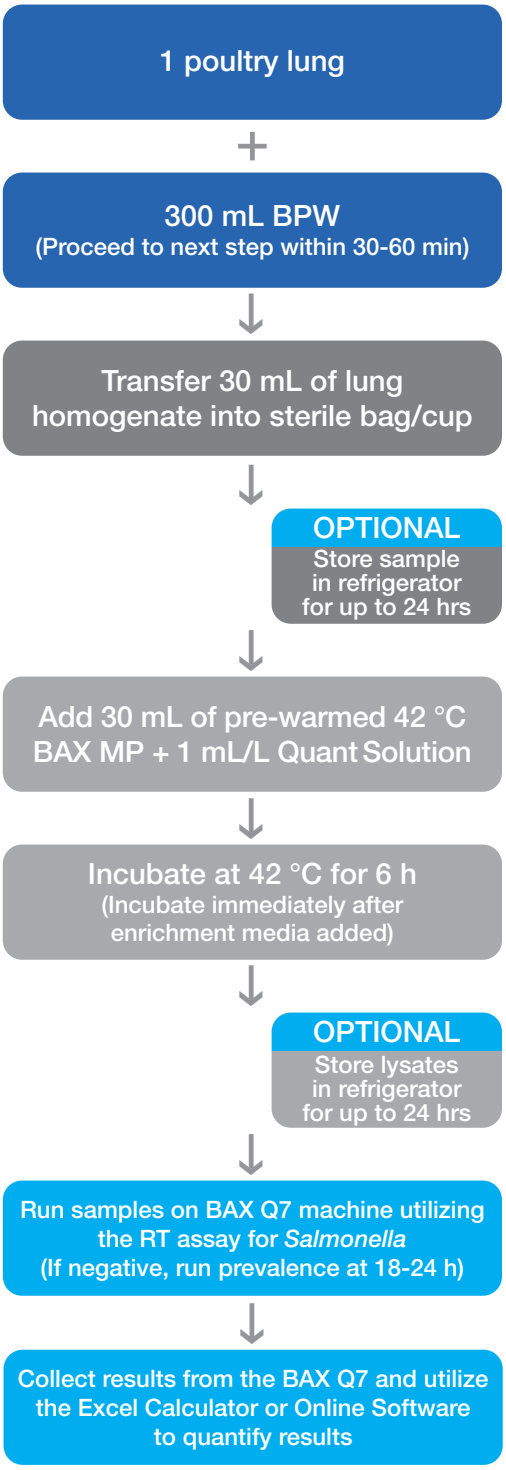
Add 1 poultry lung to 300 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

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

Incubate sample at 42 ± 1 °C for 6 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Poultry
Primary Production

Poult Pads (cardboard or straw) - *Salmonella*

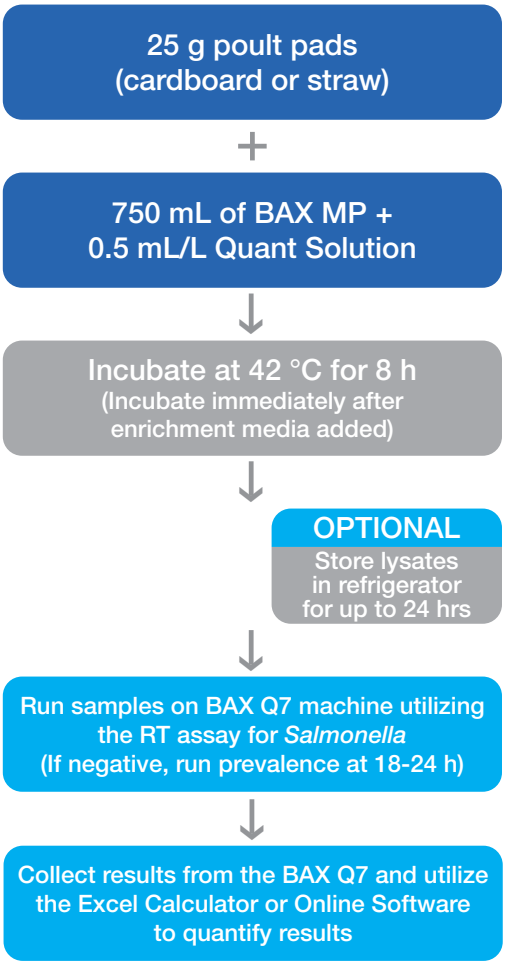
Enrichment & PCR Procedure

Add 25 g of poult pads (cardboard or straw) to 750 mL of pre-warmed (42 °C) BAX MP media containing 0.5 mL/L of BAX Quant Solution as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1 °C for 8 h.

Following 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. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for the remainder of the 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Poultry
Primary Production

Spleen - *Salmonella*

Enrichment & PCR Procedure

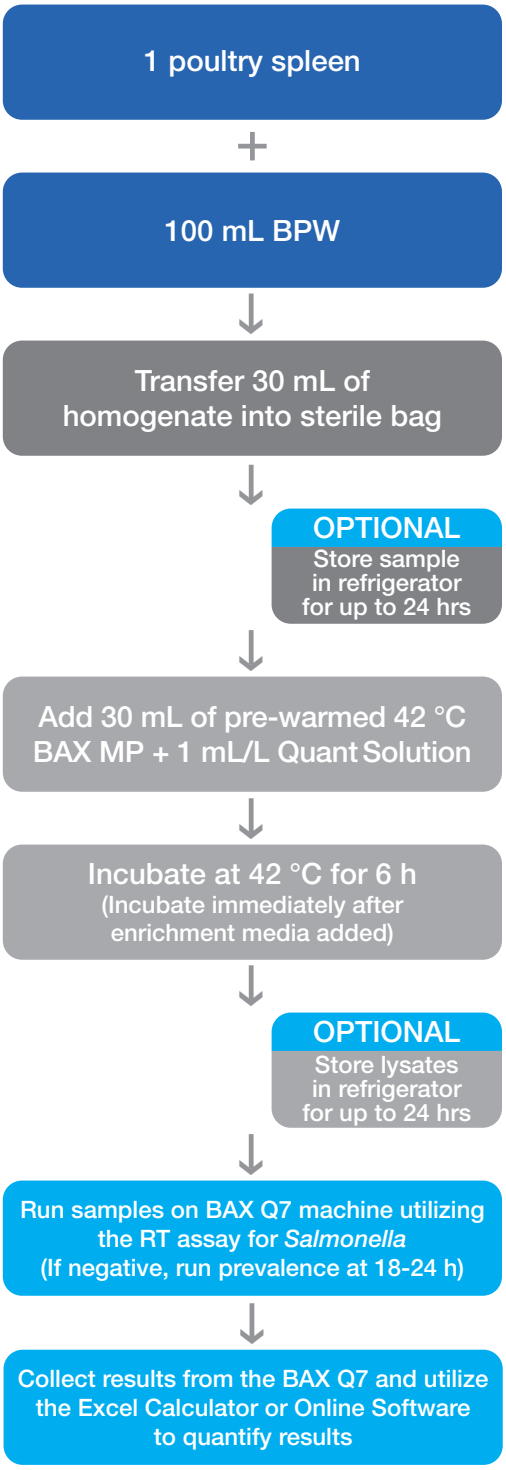
Add 1 poultry spleen to 100 mL of Buffered Peptone Water (BPW) as the Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

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

Incubate sample at 42 ± 1 °C for 6 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Poultry Processing

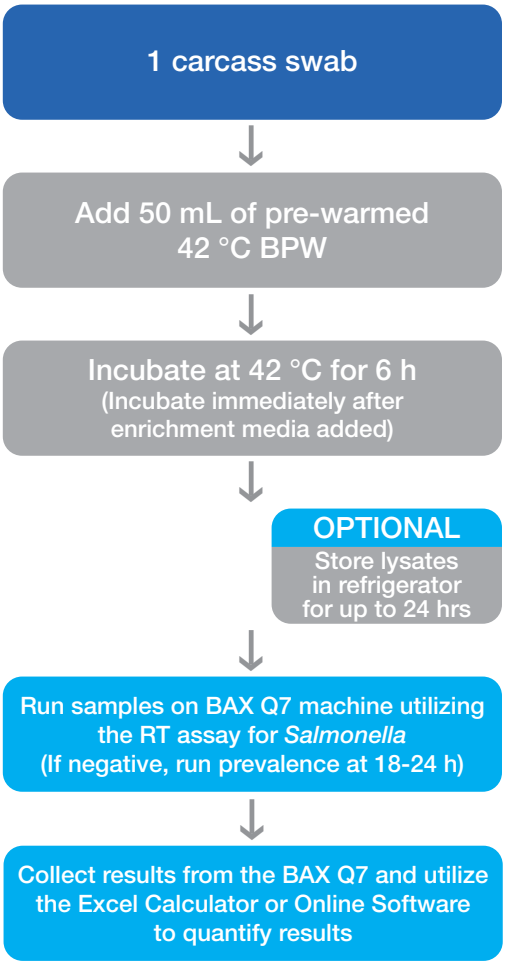
Poultry Carcass Swabs - *Salmonella* Enrichment & PCR Procedure

Add 1 carcass swab to 50 mL of pre-warmed (42 °C) BPW media as Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

Incubate sample at 42 ± 1 °C for 6 h.

Following 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. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for remainder of 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Poultry Processing

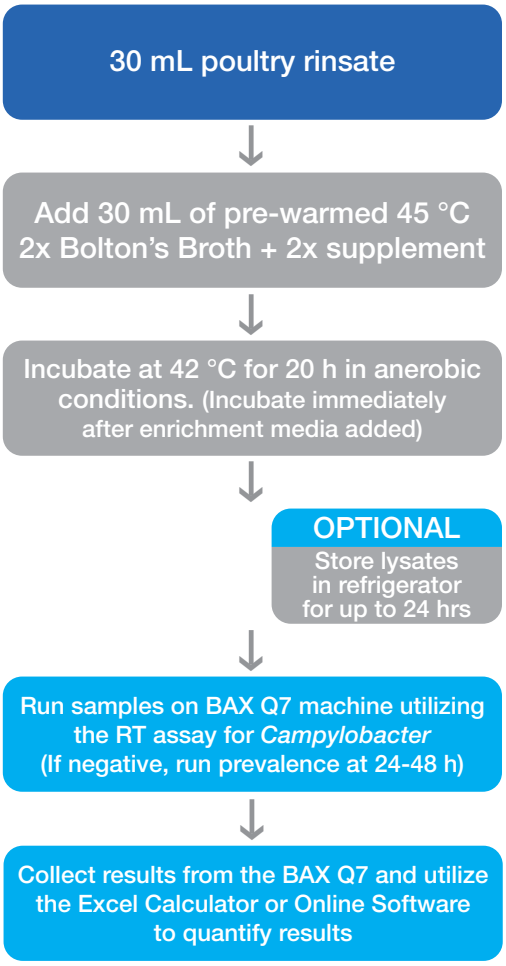
Rinsate, Carcass or Parts - *Campylobacter* Enrichment & PCR Procedure

Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (45 °C) 2X Bolton's Broth + 2X supplement. Hand massage for 15-30 seconds for homogenization.

Incubate sample at 42 ± 1 °C for 20 h in anerobic conditions.

Following enrichment for quantification, perform PCR analysis with the BAX System Real-Time PCR Assay for *Campylobacter jejuni/coli/lari*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for remainder of the 24-48 h for prevalence testing using the BAX System Real-Time PCR Assay for *Campylobacter jejuni/coli/lari*.



Poultry Processing

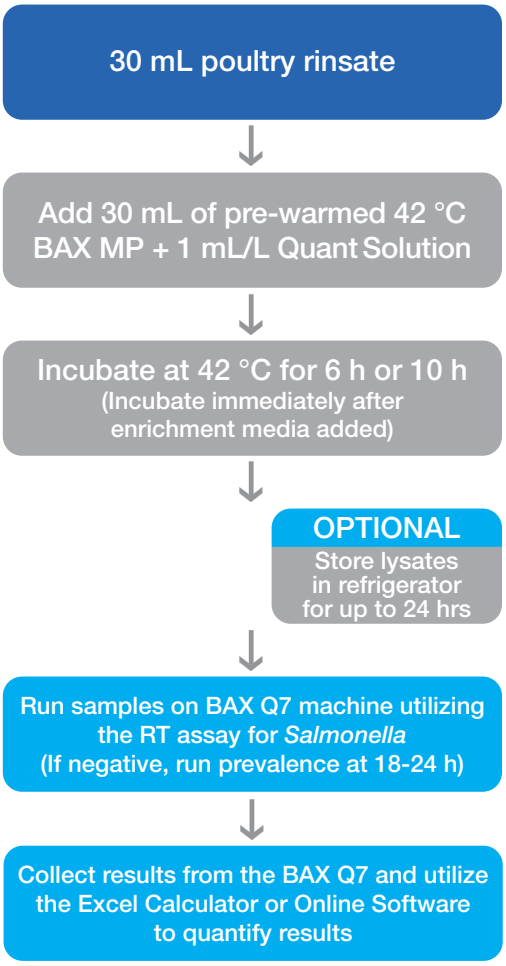
Rinsate, Carcass or Parts - *Salmonella* Enrichment & PCR Procedure

Rinse 1 poultry carcass or 4 lbs. (1.8 kg) of parts in 400 mL of BPW or nBPW. Add 30 mL of carcass or parts rinsate to 30 mL of pre-warmed (42 °C) BAX MP media containing 1 mL/L of BAX Quant Solution. Hand massage for 15-30 seconds for homogenization.

Incubate sample at 42 ± 1 °C for 6 h for 1 - 10,000 CFU/mL enumerable range, or 10 h for 1 - 3 CFU/30 mL enumerable range.

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. View more instructions for calculators on pages 46-50.

After quantification enrichment, continue incubation of the Primary Enrichment for remainder of the 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Salmonella*.



Poultry
Final Product

Comminuted Poultry - *Salmonella*

Enrichment & PCR Procedure

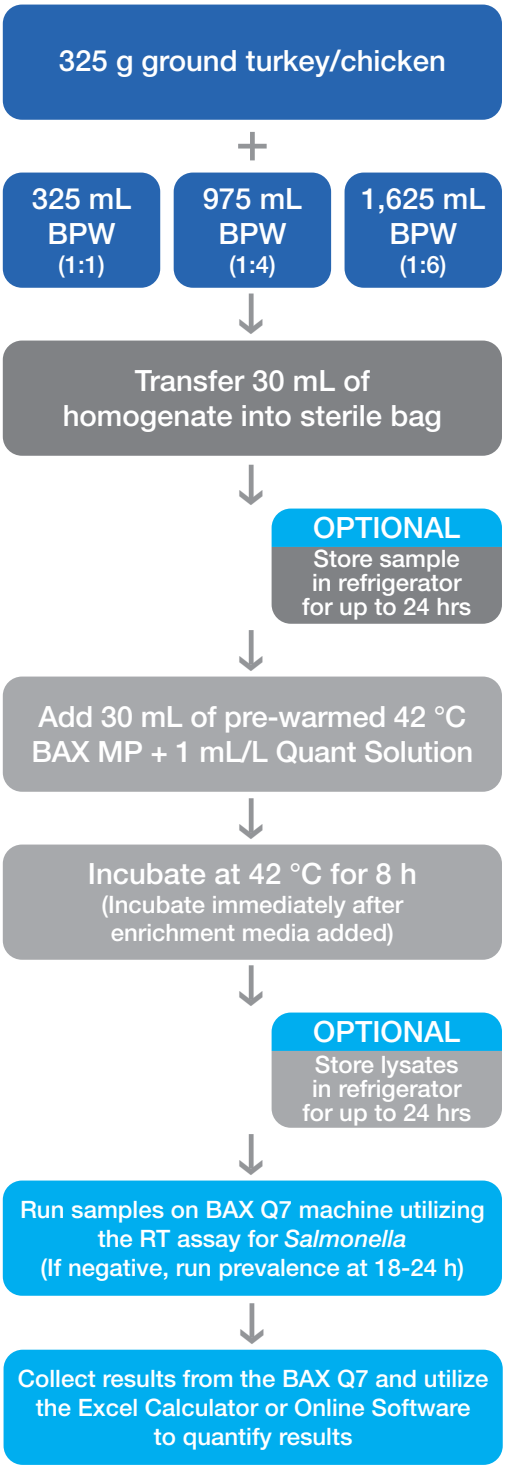
Add 325 g of comminuted chicken or turkey to 325 mL (1:1), 975 mL (1:4), or 1,625 mL (1:6) of Buffered Peptone Water (BPW) as the Primary Enrichment. (**AOAC *Performance Tested*℠ 081201**) Homogenize at 230 RPM for 30 seconds.

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

Incubate sample at 42 ± 1 °C for 8 h.

Following 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. View more instructions for calculators on pages 46-50.

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*.



Seafood
Final Product

Oysters - *Vibrio*

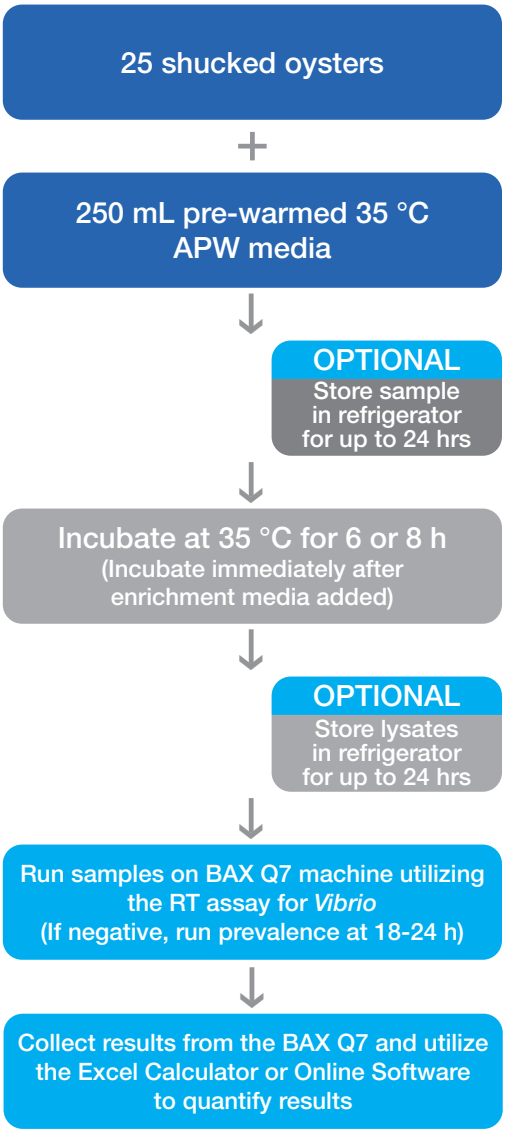
Enrichment & PCR Procedure

Add 1 MicroTally to 250 mL of pre-warmed (42 °C) Alkaline Peptone Water (APW) media as Primary Enrichment. Homogenize at 230 RPM for 30 seconds.

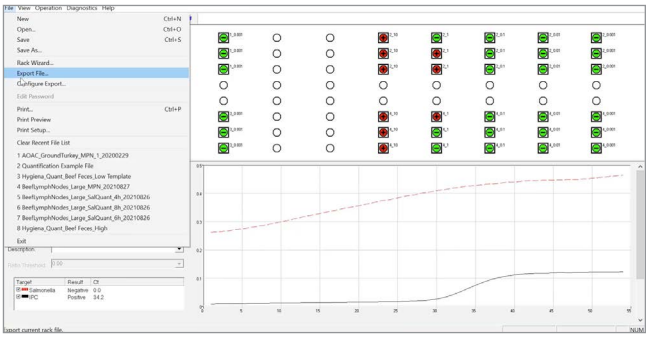
Incubate sample at 42 ± 1 °C for 6 h for LOD10 or 8 h for LOD1 and 1 – 10,000 CFU/mL enumerable range.

Following enrichment for quantification or LOD, perform PCR analysis with the BAX System Real-Time PCR Assay for *V. cholerae, V. parahaemolyticus, V. vulnificus*. Collect results from the BAX System Q7 and utilize the Excel Calculator or Online Software to quantify results. View more instructions for calculators on pages 46-50.

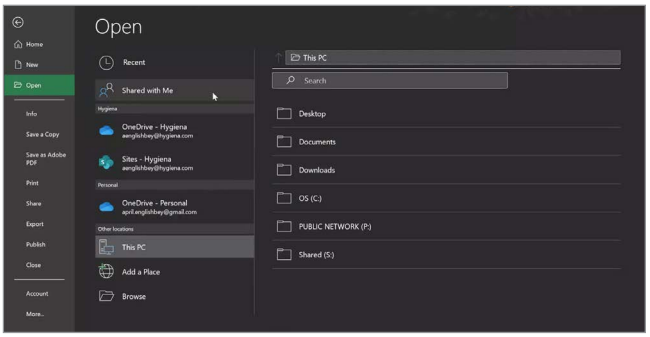
After quantification enrichment, incubate the remaining Primary Enrichment at 37 ± 1 °C for 18-24 h for prevalence testing using the BAX System Real-Time PCR Assay for *Vibrio*.



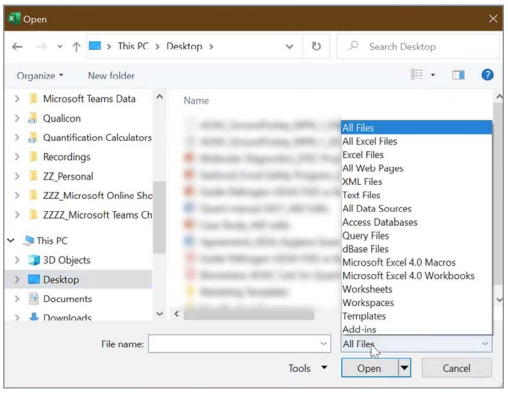
1. Following the completion of the BAX System Real-Time PCR result analysis, export the file as a .txt file. FILE > Export File > Save in a known location as .txt file.



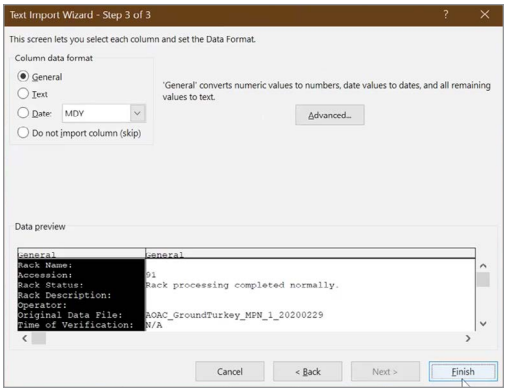
2. Open the Microsoft Excel application.



3. Within the Excel application > Select Open File > Navigate to .txt file location > Change file type to All Files > Select .txt file.

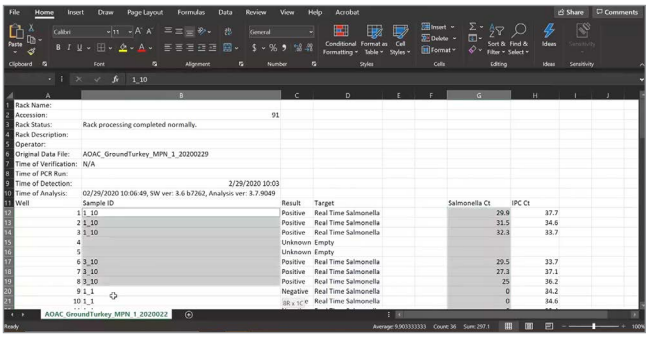


4. Select Next on Text Import Wizard for all steps, then select Finish.



MicroSoft Excel Calculator (continued)

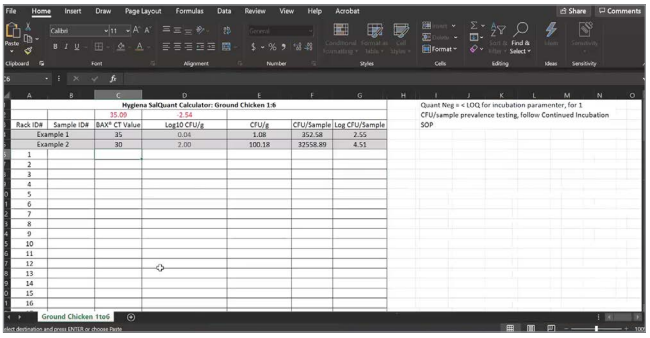
5. Select and copy Sample ID and Organism CT Value columns.



6. Open specified Excel calculator that is specific to matrix and enrichment protocol being tested.

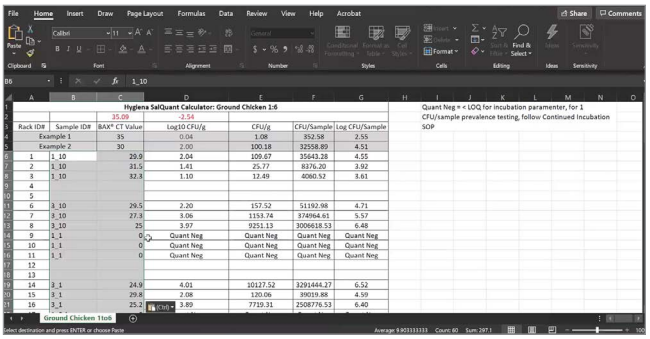
   Note: Using the wrong calculator will result in miscalculation of results.

   Visit this site to request the specified Excel calculator.



7. Paste Sample ID and Organism C Value into Excel calculator specific to matrix and enrichment protocol.

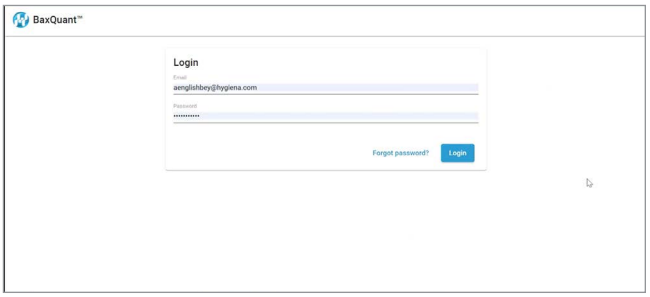
   Note: Steps 1-5 can be skipped by manually entering information into Excel calculator instead of exporting file.



8. Calculator will auto-calculate results in multiple unit conversions to utilize at user's discretion.

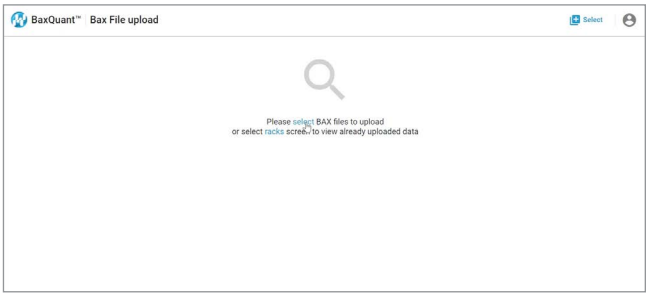
For questions or concerns, please contact:
Diagnostics Support at **diagnostics.support@hygiena.com**

1. Following the completion of the BAX System Real-Time PCR result analysis, ensure file is saved in a known location as a .bax file.



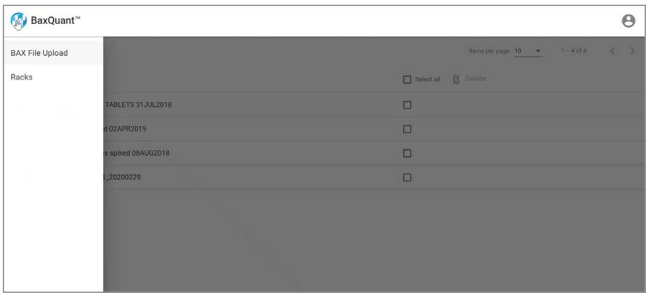
2. Log into the Quant Online portal.

   Note: User accounts can be requested from Diagnostics Support at **diagnostics.support@hygiena.com**.



3. Click Select to upload BAX files.

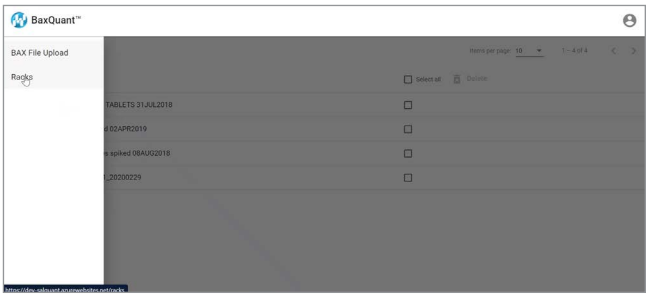
   Note: If additional files need to be uploaded, select the Hygiena logo in the top left corner of screen. A panel will open to Select BAX File Upload.



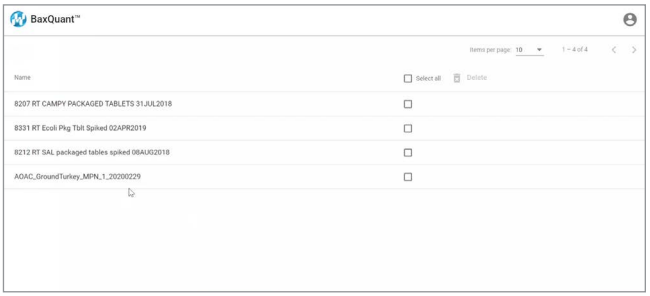
Quant Online (continued)

4. Following upload, select Racks to view uploaded data.

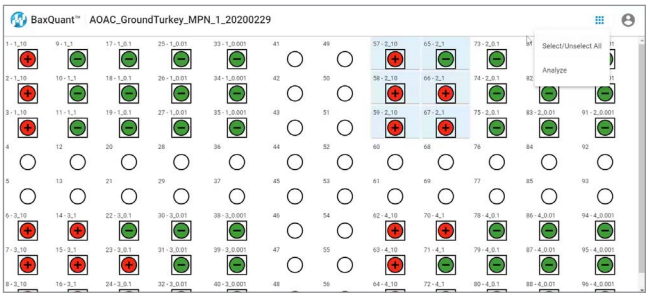
   Note: If files have previously been uploaded, select the Hygiena logo in the top left corner of screen. A panel will open to select Racks to view available data. Data will only maintain within system for 5 days and can be manually deleted by selecting the box and clicking delete.



5. Select file by clicking on file name to open rack.

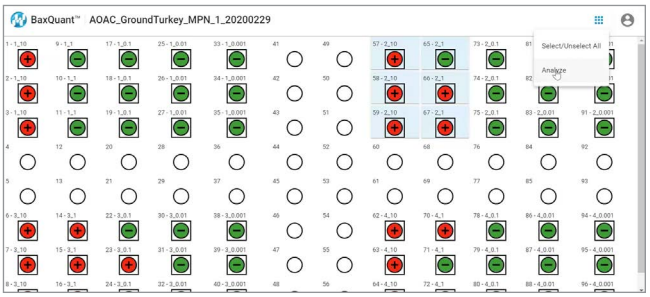


6. To select wells to perform calculation on, individually click on wells or select All/Deselect All by clicking on blue box icon in top right corner of screen.

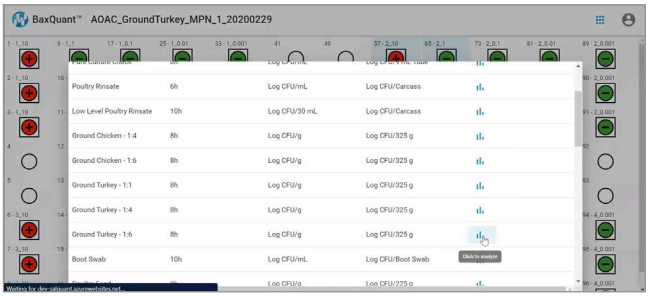


Quant Online (continued)

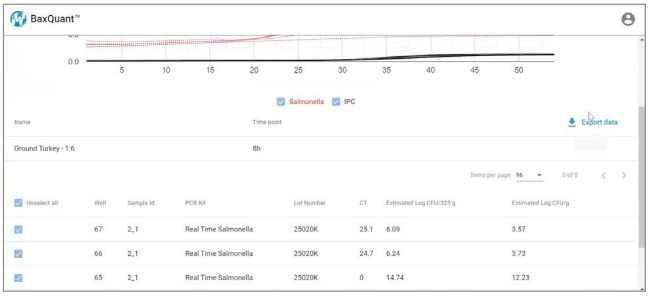
7. Once wells are selected, click blue box icon in top right corner of screen and select Analyze.



8. A window will pop up to select matrices being analyzed. Select blue bar graph icon on right side of window to start analysis and calculation.



9. Estimations will be displayed in multiple unit formats to be used at user's discretion or data can be exported as a .csv file by clicking the blue Export Data icon below PCR graphs.



For questions or concerns, please contact:
Diagnostics Support at **diagnostics.support@hygiena.com**

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**.

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