hygiena®

BAX® System Real-Time PCR Assay

Salmonella

Part KIT2006

PERFORMANCE TESTED AOAC RESEARCH INSTITUTE LICENSE NUMBER 081201

BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140

QUA 18/08-03/15
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
http://nf-validation.afnor.org/en

KIT CONTENTS

96 PCR tubes with tablets (2 bags of 6 x 8 strips)

96 flat optical caps (12 x 8 strips)

1 bottle of protease (400 µL)

2 bottles of lysis buffer (12 mL)

INTENDED USE

Food processors and associated laboratories can use the BAX® System as a quick and reliable method for detecting *Salmonella* in a variety of foods, hemp and cannabis flowers, and environmental surfaces. The real-time PCR assay was designed to report yes/no results for *Salmonella* at concentrations as low as 10^4 cfu/mL after enrichment. With a processing time of approximately 60 minutes in the BAX® System Q7 instrument, the method returns results comparable to culture methods, but with a significantly faster time to result.

With the SalQuant method application, the intended use can be expanded to include quantification of various poultry, beef and pork matrices. In addition, the real-Time PCR assay can also be used as an alternative to the traditional MPN method for raw comminuted turkey and chicken, whole carcass poultry rinses, and raw beef trim.

BAX® Systems are designed for use by qualified lab personnel who follow standard microbiology laboratory practice, including the safe handling and disposal of potentially pathogenic materials. The laboratory must comply with good laboratory practice (see ISO 7218 standard). For an NF-validation method, laboratory must follow ISO 22174 (Microbiology of food and animal feeding stuffs-Polymerase chain reaction (PCR) for the detection of food-borne pathogens--General requirements and definitions).

Field of use: Data obtained from the BAX® System should not be used for human diagnostic or human treatment purposes. Equipment is not approved by the United States Food and Drug Administration or any other U.S or non-U.S. regulatory agency for use in human diagnostics or treatment. The BAX® System should not be used as the sole basis for assessing the safety of products for release to consumers. The information generated is only to be used in conjunction with the user's regular quality assurance program. Not approved for clinical diagnosis. Use for research and development, quality assurance and quality control under supervision of technically qualified persons.

PRINCIPLE OF THE METHOD

See the BAX® System User Guide for an overview of how the BAX® System method uses automated, real-time Polymerase Chain Reaction (PCR) technology.

MATERIALS

BAX® System Real-Time PCR Assay for *Salmonella* (Part KIT2006])

BAX® System start-up package (equipment and supplies for up to 192 tests)

- BAX® System Q7 cycler/detector and computer workstation
- Heating blocks with inserts* capable of maintaining 37±2ºC and 95±3ºC
- Cooling blocks with inserts*
- PCR tube holder
- Capping/decapping tools
- Adjustable mechanical pipettes (5-50 µL; 20-200 µL)
- Repeating pipette
- Multi-channel pipette (8 channels – 5-50 µL)
- Cluster tubes with caps and racks
- Pipette tips with barriers
- Powder-free nitrile gloves

**The Automated Thermal Block (Catalog No. MCH2023) may be used in place of heating and cooling blocks.*

Stomacher with bags.

Incubator capable of maintaining directed enrichment temperatures within ±2°C.

***Note**: Health Canada, AOAC, and AFNOR Certification standards require an incubator capable of maintaining ±1°C.*

Enrichment media. (See BAX® System User Guide for details)

***Note**: For an NF-Validation method, please note that for the preparations of master solutions, you must follow the instructions from the EN ISO 6887 standards.*

STORAGE AND SHELF LIFE

- Reagents and PCR tubes with tablets should be kept refrigerated at 2–8°C. Do not freeze.
- Reagents should be used by the expiration date stamped on the individual labels.
- After protease has been added to the lysis buffer, shelf life of the solution is 2 weeks when stored at 2-8°C.
- If storing PCR tubes with tablets in an open kit for more than 3 weeks, seal the Mylar bag of PCR tubes into a larger bag with desiccant or store at 4ºC in a desiccation unit, if possible.

SAFETY PRECAUTIONS

The BAX® System method includes sample enrichment procedures that nourish the growth of potential pathogens to detectable levels. Because pathogens can cause human illness, appropriate safety precautions must be taken and personal protective equipment worn 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 assay, please review the Safety Data Sheets (SDS) included with your BAX® System purchase and also available at www.hygiena.com. Refer to your site practices for safe handling of materials at extreme temperatures.

SOFTWARE REQUIREMENTS

Before using this assay for the first time, install the most current version of the BAX® System software, then run a calibration report to check that "Real Time *Salmonella*" appears in the list of calibration files. See "Troubleshooting Calibration" in the BAX® System User Guide for details.

If the report list does not contain "Real Time *Salmonella*", you must recalibrate the Q7 instrument to load the required dyes. Be sure to allow enough time to complete the calibration (about 1.5 to 2 hours) before starting the assay. For instructions and tips on calibrating the instrument, see the BAX® System User Guide.

ENRICHMENT PROTOCOL - STANDARD ENRICHMENT MEDIA

1. **Prepare Enrichment Broth**
   Prepare enrichment broth according to the manufacturer's instructions. See the BAX® System User Guide for common enrichment media recipes.

2. **Collect and Enrich Samples**

   Method Approved by AOAC

- *Ground beef*: **For 25 g** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. **For 375 g** – Homogenize 375 g sample with 1.5 L pre-warmed (45-46°C) mTSB with 2 mg/L novobiocin. Incubate at 39-42°C for 22-26 hours.
- *Ground beef with soy*: **For 25 g** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. **For 325 g** – Homogenize 325 g sample with 975 mL pre-warmed (35°C) mTSB with 10 g/L casamino acids and 8 mg/L novobiocin. Incubate at 35°C for 20-24 hours.
- *Beef trim:* **For 25 g** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 20-24 hours. **For 325 g** – Homogenize 325 g sample with 1.5 L pre-warmed (41°C) BAX® System MP media. Incubate at 39-42°C for 16-24 hours.
- *Poultry rinses*: Homogenize 30 mL BPW sample rinsate with 30 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours.
- *Ground turkey and chicken wings*: Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 16-24 hours.
- *Frankfurters:* Homogenize 325 g sample with 1400 mL pre-warmed (35°C) BPW. Add additional BPW to reach a total media volume of 2925 mL. Incubate at 35°C for 18-24 hours.
- *Shrimp:* Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours.
- *Shell eggs:* Combine 20 eggs (~1000 mL) into sterile container with 2 L pre-warmed (42°C) BAX® System MP media. Incubate at 42°C for 48 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Eggs, Dried*: **LB Enrichment** – Add approximately 15 mL pre-warmed (35°C) LB to 25 g sample and stir to smooth. Add 3 additional aliquots of LB of 10 mL, 10 mL, and 190 mL (total media volume 225 mL), stirring after each addition. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **BPW Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Peanut butter*: **LB Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours.

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Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **BPW Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.

- *Lettuce*: **MP Media Enrichment** – Add 25 g sample to 225 mL pre-warmed (35°C) BAX® System MP media and swirl 25 times clockwise and 25 times counterclockwise to mix. Incubate at 35°C for 10-24 hours. **LB Enrichment** – Add 25 g sample to 225 mL pre-warmed (35°C) LB and swirl 25 times clockwise and 25 times counterclockwise to mix. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours.
- *Frozen Peas*: **MP Media Enrichment** – Homogenize sample with 225 mL pre-warmed (35°C) BAX® System MP media. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **LB Enrichment** – Homogenize sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Cream cheese*: **MP Media Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BAX® System MP media. Incubate at 35°C for 12-24 hours. **LB Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2. Incubate at 35°C for 22-26 hours.
- *Nonfat dry milk*: Pour 25 g sample slowly over the surface of 225 mL pre-warmed (35°C) Brilliant Green Water. Let stand at room temperature for 55-65 minutes. Do not mix or adjust pH. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Ice cream:* **LB Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **BPW Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) BPW. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **BGW Enrichment** – Homogenize 25 g sample with 225 mL pre-warmed (35°C) Brilliant Green Water. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Milk-based infant formula:* Homogenize 25 g sample with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Cocoa:* Homogenize 25 g sample with 225 mL reconstituted nonfat dry milk. Let stand at room temperature for 55-65 minutes, then swirl thoroughly to mix. Adjust pH to 6.8 ± 0.2, if necessary. Add 0.45 mL 1% aqueous brilliant green dye solution and mix well. Incubate at 35°C for 22-26 hours. Transfer 10 µL enrichment to 500 µL BHI broth before processing. *Optional* - Incubate BHI broth at 37°C for 3 hours.
- *White pepper:* Homogenize 25 g sample with 225 mL pre-warmed (35°C) TSB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Orange Juice*: **MP Media Enrichment** – Swirl 25 mL sample thoroughly with 225 mL pre-warmed (41°C) BAX® System MP media. Incubate at 39-42°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **UPB Enrichment** – Swirl 25 mL sample thoroughly with 225 mL pre-warmed (35°C) UPB. Let stand at room temperature for 55-65 minutes. Do not mix or adjust pH. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Dry pet food:* **LB Enrichment** – Homogenize 375 g sample with approximately one-third to one-half of 3375 mL pre-warmed (35°C) LB. Add the remainder of the pre-warmed media. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **BPW Enrichment** – Homogenize 375 g sample with approximately one-third to one-half of 3375 mL pre-warmed (35°C) BPW. Add the remainder of the pre-warmed media. Incubate at 35°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Environmental sponges (Stainless steel, ceramic tile, plastic):* **LB Enrichment** – Homogenize sponge with 225 mL pre-warmed (35°C) LB. Let stand at room temperature for 55-65 minutes. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 22-26 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours. **BPW Enrichment** – Homogenize sponge with 225 mL pre-warmed (35°C) BPW. Adjust pH to 6.8 ± 0.2, if necessary. Incubate at 35°C for 18-24 hours. *Optional* - Transfer 10 µL enriched sample to 500 µL pre-warmed (37°C) BHI. Incubate at 37°C for 3 hours.
- *Dried cannabis and hemp flower*: Combine 10 g flower with 90 mL prewarmed (37-42°C) BPW. Hand massage for 1 minute and incubate at 42°C for 22-26 hours. Add 10 µL enrichment to 500 µL prewarmed BHI broth (37ºC) and incubate in 37°C heat block for 3 hours.
- *Sampling Cloths such as MicroTally® (375 g Beef Trim)*: **MP media Enrichment**: Combine one sampling cloth with 200 mL pre-warmed (42°C) MP media. Stomach for 1 minute and incubate at 42 ± 1°C for 10-24 hours. **mTSB + caa Enrichment**: Combine one sampling cloth with 200 mL pre-warmed (42°C) mTSB + caa (modified TSB with casamino acids) media. Stomach for 1 minute and incubate at 42 ± 1°C for 8-24 hours.

ENRICHMENT PROTOCOL – ACTERO™ ENRICHMENT MEDIA

1. **Prepare enrichment broth**

   Prepare Actero™ Elite *Salmonella* Enrichment Media according to the manufacturer's instructions.

2. **Collect and enrich samples**

- *Milk Chocolate*: Homogenize 25 g sample with 175 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 22-26 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Chocolate Liquor:* Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 26-30 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Dry pet food*: **25 g sample -** Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ *Salmonella* Enrichment Media. Incubate at 35°C for 18-22 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing. **375 g sample** – Homogenize 375 g sample with approximately one-third to one-half of 2625 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Add the remainder of the pre-warmed media. Incubate at 35°C for 18-22 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Cocoa:* Homogenize 25 g sample with 175 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 5% NFDM. Incubate at 35°C for 16-20 hours. Transfer 40 µL enrichment to 2 mL PBS and mix before processing.
- *Shell Eggs:* Homogenize by hand a 20 egg sample with 1000 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours. Transfer 40 µL enrichment to 2 mL PBS and mix before processing.
- *Ground Chicken:* Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 50 mg/L of malachite green. Incubate at 35°C for 14-18 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Ground Beef:* **25 g sample -** Homogenize 25 g sample with 75 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 50 mg/L of malachite green. Incubate at 35°C for 16-20 hours. **375 g sample** – Homogenize 375 g sample with 1125 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 25 mg/L of malachite green. Incubate at 39 - 42°C for 20-24 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Chicken Carcass Rinse:* Homogenize by hand 30 mL of BPW rinse sample with 30 mL pre-warmed (35°C) Actero™ *Salmonella* Enrichment Media supplemented with 20 mg/L of malachite green. Incubate at 35°C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Dried Whole Egg:* Homogenize by hand 100 g sample with 600 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media supplemented with 5% NFDM. Incubate at 35°C for 14-18 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Whole Liquid Egg (Pasteurized):* Homogenize by hand 100 g sample with 300 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Adjust pH to 7.0 ± 0.2 if necessary. Incubate at 35°C for 18-22 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Raw Almond:* Homogenize 375 g sample with 750 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Dried Raisins:* Homogenize 25 g sample with 75 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Peanut Butter:* Homogenize 25 g sample with 175 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Whole Black Pepper:* Homogenize 25 g sample with 75 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 16-20 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Dried Parsley:* Homogenize 25 g sample with 225 mL pre-warmed (35°C) Actero™ Elite *Salmonella* Enrichment Media. Incubate at 35°C for 20-24 hours. Transfer 80 µL enrichment to 2 mL PBS and mix before processing.
- *Environmental sponges (Stainless steel and plastic)*: Homogenize sponge with 90 mL pre-warmed (35°C) Actero™ *Salmonella* Enrichment Media. Incubate at 35°C for 14-18 hours. Transfer 40 µL enrichment to 2 mL PBS and mix before processing.

ENRICHMENT PROTOCOL- BAX® System SalQuant™

1. **Prepare Enrichment Broth**

- BAX® System MP Media–Prepare MP media according to manufacturer's instructions.
- BAX® System MP Media plus Quant Solution –Prepare MP media according to manufacturer's instructions.

Autoclave at 121°C for 15 minutes, then add 1 mL Quant Solution per liter.

- *Raw comminuted Turkey and Chicken:* Add 325 g portions to 1625 mL BPW. Homogenize by hand until all clumps have been dispersed. Transfer 30 mL of homogenate to a sterile filtered bag. Add 30 mL of prewarmed (42°C) BAX® MP media with novobiocin (40 mg/L) to each sample bag and hand mix for 30 seconds. Incubate samples at 42 ± 1°C for 8 h. After transferring to BAX® MP with novobiocin for BAX® System SalQuant™ enrichment, incubate the remaining original homogenate sample at 35°C for 24 hours for prevalence testing.
- *Whole Bird Rinsates:* Combine 30 mL poultry rinsate to 30 mL prewarmed (45°C) BAX® MP media plus 1 mL/L Quant Solution. Hand mix for 30 seconds and incubate sample at 42°C for 6 hours. After pulling aliquot at the determined timepoint for lysate creation, incubate the remaining original homogenate sample at 42°C for 18-24 hours for prevalence testing.
- *Raw Ground Beef and Raw Ground Pork:* Add 375 g portions to 1.5 L prewarmed (45°C) BAX® MP media and hand massage until all clumps have been dispersed. Transfer 30 mL of homogenate to a sterile filtered bag. Add 30 mL of prewarmed (45°C) BAX® MP media plus 1 mL/L Quant Solution. Hand mix for 30 seconds and incubate sample at 42°C for 6 hours for raw ground beef and at 7 hours for raw ground pork. Incubate the remaining original homogenate sample (375 g in 1,500 mL BAX MP) at 42°C for 18-24 hours for prevalence testing.
- *Raw Beef Trim and Raw Pork Trim:* Add 375 g portions to 1.5 L prewarmed (45°C) BAX® MP media and hand massage for 30 seconds. Incubate sample at 42°C for 6 hours. After pulling aliquot at the determined timepoint for lysate creation, incubate the remaining original homogenate sample at 42°C for 18-24 hours for prevalence testing.
- *MicroTally on Raw Beef Trim and MicroTally on Raw Pork Trim:* Add one MicroTally cloth to 200 mL prewarmed (45°C) BAX® MP media and hand mix for 30 seconds. Incubate sample at 42°C for 6 hours. After pulling aliquot at the determined timepoint for lysate creation, incubate the remaining original homogenate sample at 42°C for 18-24 hours for prevalence testing.

ENRICHMENT PROTOCOL FOR MPN ESTIMATION

- *Raw comminuted Turkey and Chicken:* Homogenize 65 g samples with 585 mL BPW. Make 3-tube 5-dilution MPN set representing 1 g, 0.1 g, 0.01 g, 0.001 g, and 0.0001 g of sample by setting up the following: For 1 g sample dilution, fill 3 test tubes with 10 mL homogenate. For 0.1 g sample dilution, fill 3 test tubes of 9 mL BPW with 1 mL sample homogenate. For 0.01 g sample dilution, fill 3 test tubes of 9.9 mL of BPW with 0.1 mL of sample homogenate. For 0.001 g sample dilution, add 0.1 mL of sample homogenate to 9.9 mL BPW, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. For 0.0001 g sample dilution, add 0.1 mL of homogenate to 99.9 mL BPW, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. Incubate tubes at 37°C for 24 hours. Continue with creation of lysates for each incubated tube for prevalence testing.
- *Whole Bird Rinsates:* Make 3-tube 5-dilution MPN set representing 1 g, 0.1 g, 0.01 g, 0.001 g, and 0.0001 g of sample by setting up the following: For 1 mL sample dilution, fill 3 test tubes with 1 mL rinsate and 9 mL BPW. For 0.1 mL sample dilution, fill 3 test tubes of 9 mL BPW with 1 mL from previous sample dilution. For 0.01 mL sample dilution, fill 3 test tubes of 9 mL of BPW with 1 mL from previous sample dilution. For 0.001 ml sample dilution, add 1 mL from previous sample dilution to 9 mL BPW. For 0.0001 mL sample dilution, add 1 mL from previous sample dilution to 9 mL BPW. Incubate tubes at 37°C for 24 hours. Continue with creation of lysates for each incubated tube for prevalence testing.
- *Raw Beef Trim:* Homogenize 65 g samples with 585 mL mTSB and hand mix for 30 seconds. Make 3-tube 5-dilution MPN set representing 1 g, 0.1 g, 0.01 g, 0.001 g, and 0.0001 g of sample by setting up the following: For 1 g sample dilution, fill 3 test tubes with 10 mL homogenate. For 0.1 g sample dilution, fill 3 test tubes of 9 mL mTSB with 1 mL sample homogenate. For 0.01 g sample dilution, fill 3 test tubes of 9.9 mL of mTSB with 0.1 mL of sample homogenate. For 0.001 g sample dilution, add 0.1 mL of sample homogenate to 9.9 mL mTSB, vortex, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL BPW. For 0.0001 g sample dilution, add 0.1 mL of homogenate to 99.9 mL mTSB, then add 1.0 mL from this dilution to 3 tubes containing 9.0 mL mTSB. Incubate tubes at 42°C for 24 hours. Continue with creation of lysates for each incubated tube for prevalence testing.

Method Approved by AFNOR Certification

Test portions weighing more than 25 g have not been tested in the context of NF VALIDATION.

For preparation of initial suspensions, follow instructions of EN ISO 6579 and EN ISO 6887 standards.

- *General Protocol for meat products (including meat with spices or herbs), seafood, vegetable, pet food, environmental samples*: Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37°C for 16-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37°C for 3-4 hours.
- *Egg products*: Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37°C for 18-24 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37°C for 3-4 hours.
- *Raw beef (short protocol):* Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 41.5°C for 10-24 hours.
- *Raw Meats and raw seafood:* Homogenize 25 g sample with 225 mL pre-warmed BPW. Incubate at 37°C for 16-20 hours.
- *Dairy Products (except powdered milk):* Homogenize 25 g sample with 225 mL pre-warmed BPW with 20mg/L novobiocin. Incubate at 41.5°C for 20-24 hours.
- *Chocolate:* Homogenize 25 g sample with 225 mL pre-warmed NFDM (reconstituted non-fat dried milk). Let stand for 55-65 minutes. Add 450 µL of 1% Brilliant Green. Incubate at 37°C for 22-26 hours. Transfer 10 µL enriched sample to 500 µL pre-warmed BHI broth. Incubate at 37°C for 3-4 hours.

  Note: *Follow General Protocol, for matrices not falling into protocols listed above*

  Note*: Due to the sensitivity of short enrichment protocols, it is important that incubation times and temperatures are followed as closely as possible. Verify that media is sufficiently pre-warmed to incubation temperature before adding samples, and that the delay between pre-warming media and adding samples does not exceed 45 minutes. Use of a ventilated incubator is recommended.*

TEST PROTOCOL

3. Prepare Equipment

3.1 Turn on the heating blocks to 37ºC and 95ºC*.

3.2 Make sure cooling blocks are chilled to 2-8ºC*.
**If using the Automated Thermal Block, follow the instructions in the Automated Thermal Block User Guide for running the Gram Negative program.*

3.3 Power on the Q7 instrument and launch the BAX® System application.

3.4 Create a rack file (see User Guide for details).

4. Perform Lysis

4.1 Break cluster tubes apart.

4.2 Label and arrange cluster tubes in rack according to the rack file.

4.3 Prepare lysis reagent by adding 150 µL protease to one 12 mL bottle of lysis buffer.

4.4 Transfer 200 µL lysis reagent to each cluster tube.

4.5 Transfer 5 µL enriched sample to the corresponding cluster tube.

Note*: Enrichments can be stored at room temperature until test results have been reviewed and accepted (up to 4 hours unless otherwise validated internally).*

4.6 Heat at 37°C for 20 minutes.

4.7 Heat at 95°C for 10 minutes.

4.8 Cool at 2-8°C for at least 5 minutes.

5. Hydrate PCR Tablets

5.1 Initialize the instrument by selecting RUN FULL PROCESS from the OPERATION menu.

5.2 Place a PCR tube rack onto a chilled (2-8°C) PCR cooling block.

5.3 Arrange strips of PCR tubes according to your rack file.

5.4 Remove the caps from the first strip of tubes with the decapping tool.

5.5 Transfer 30 µL lysate (from step 4.8) into PCR tubes, then seal with flat optical caps.

5.6 Repeat with remaining strips of PCR tubes until all PCR tablets have been hydrated.

5.7 Let PCR tubes sit in the cooling block for 10-30 minutes before loading into the BAX® System instrument.

Note*: Do not let PCR tubes sit for more than 30 minutes.*

6. Amplify and Detect

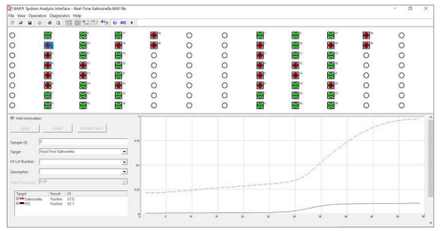
6.1 At the “Ready for Rack Load” prompt, click the NEXT button and open the instrument drawer.

6.2 Place the rack of PCR tubes over the wells in the drawer, and check that the tubes are seated correctly.

6.3 Close the drawer and click the NEXT button to begin automated processing.

7. Review Results

Qualitative results are displayed as a grid of color-cued icons in the top half of the screen:



Green (-)	=	Negative for target organism
Red (+)	=	Positive for target organism
Yellow (?)	=	Indeterminate result*
Yellow (?) with red slash	=	Signal error *

*Refer to the troubleshooting section in the User Guide for assistance.

FOR BAX® System SalQuant™ ONLY

Transfer CT values generated into Hygiena Quant Calculator to auto-calculate the Log_{10}CFU/g, providing a quantitative result. (Please contact Hygiena representative for training and Hygiena Quant Calculator).

FOR BAX® MPN method

Utilize MPN tables in USDA-FSIS MLG 2.05 Most Probable Number Procedure and Tables to estimate MPN/g.

CONFIRMATION

Method Approved by AOAC

If desired, BAX® method enriched samples can be confirmed with the reference culture method appropriate for the sample type, such as:

- U.S. FDA Bacteriological Analytical Manual (BAM)
- USDA FSIS Microbiology Laboratory Guidebook (MLG)
- Health Canada Compendium of Analytical Methods
- International Organization for Standardization (ISO)
- AOAC SMPR 2020.002

***Note**: For confirmation methods that require an additional plating media of choice, Oxoid Brilliance™ Salmonella plates (Oxoid PO5098A or Oxoid CM1092 & SR 0194) are recommended.*

Method Approved by AFNOR Certification

All samples identified as positive by the BAX® System method must be confirmed in one of the following ways:

- Using the conventional testing methods described by CEN or ISO, including purification.
- For General protocol (including meat with spices/herbs) and Egg products: Streak 10 µL of last enrichment to Brilliance Salmonella Agar and XLD and incubate at 37°C for 22-26 hours. To subculture, transfer 100 µL of last enrichment to 10 mL RVS broth and incubate 41.5°C for 21-27 hours. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and XLD Agar and incubate at 37 ± 1°C for 22-26 hours. Confirm presumptive positive colonies with a latex test (Oxoid).
- For Raw Beef (specific short protocol), Raw Meats and Raw Seafood: Streak 10 µL of BPW onto Brilliance *Salmonella* Agar and XLD and incubate 22-26 hours at 37 ± 1°C. To subculture, transfer 100 µL BPW enrichment to 10 mL of RVS broth and incubate 21-27 hours at 41.5°C. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and XLD and incubate at 37 ± 1°C for 22-26 hours. Confirm presumptive positive colonies with a latex test (Oxoid).
- For Dairy Products (except Powdered Milk): Streak 10 µL of BPW with novobiocin onto Brilliance *Salmonella* Agar and XLD and incubate 22-26 hours at 37 ± 1°C. To subculture, transfer 100 µL BPW with novobiocin enrichment to 10 mL of RVS broth and incubate 21-27 hours at 41.5°C. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and XLD and incubate at 37 ± 1°C for 22-26 hours. Confirm presumptive positive colonies with a latex test (Oxoid).
- For Chocolates: Streak 10 µL of last enrichment to Brilliance Salmonella Agar and XLD and incubate at 37°C for 22-26 hours. To subculture, transfer 100 µL NFDM or BHI enrichment to 10 mL RVS broth and incubate 41.5°C for 21-27 hours. Streak 10 µL of the RVS enrichment to Brilliance *Salmonella* Agar and incubate at 37 ± 1°C for 22-26 hours; streak an additional 10 µL RVS enrichment to XLD Agar and incubate at 37 ± 1°C for 18-24 hours. Confirm presumptive positive colonies with a latex test (Oxoid).

Some strains of *Salmonella* belonging to the serovar Dublin, may show weak magenta pigmentation, because of their low esterase activity.

BPW and NFDM enrichments may be stored at 2-8°C for up to 72 hours after enrichment to allow for confirmation of PCR positive results and in case of a need to repeat the PCR analysis.

In the event of discordant results (positive by the alternative method and not confirmed by one of the means described above) the laboratory must follow the necessary steps to ensure the validity of the result obtained.

DISPOSAL

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

VALIDATION

The BAX® System Real-Time PCR Assay for *Salmonella* has been certified by the AOAC Research Institute as *Performance Tested Method*℠ #081201. This test kit's performance was reviewed by AOAC-RI and was found to perform to the manufacturer's specifications. Validation studies for foods, hemp and cannabis flowers, and surfaces demonstrated BAX® System sensitivity and specificity equal to or better than the reference culture-based methods. Validation studies using the SalQuant method on raw comminuted turkey and chicken, whole bird rinses, raw ground beef and pork, raw beef and pork trim and Microtally on raw beef and pork trim, were comparable to USDA/FSIS MLG 2.05.

The BAX® System Real-Time PCR Assay for *Salmonella* has been certified as an AOAC INTERNATIONAL Official Method of Analysis (OMA) #2013.02 for detecting *Salmonella* in a variety of foods and environmental surfaces. Validation studies were performed on raw ground beef, beef trim, frankfurters, shrimp, ground turkey, chicken wings, poultry rinses, whole powdered dried eggs, shell eggs, fresh bagged lettuce, frozen peas, orange juice, cream cheese, non-fat dry milk, ice cream, peanut butter, cocoa, white pepper, milk-based infant formula, dry pet food, and environmental sponges on stainless steel, ceramic tile and plastic surfaces.

The BAX® System Real-Time PCR Assay for *Salmonella* has been certified as #QUA 18/08-03/15 according to NF VALIDATION rules. Validation studies conducted according to ISO 16140-2 standards found this test kit's performance to satisfy the ISO 16140-2 standard and NF VALIDATION Technical rules criteria for all foods, pet food and environmental samples (excluding primary production samples and powdered milk). For more information, including validity dates, please refer to certificate QUA 18/08-03/15 available at http://nf-validation.afnor.org.

The software version approved in the scope of NF-Validation certification is disclosed in the certificate. For more information about the end of validity of the NF-Validation certification, please refer to the certificate available on the website or upon request to Hygiena representative.

TECHNICAL ASSISTANCE

For questions or comments, please contact your local distributor. You can also call 800-863-6842 in the U.S., 1-302-695-5300 outside the U.S., or email diagnostics.support@hygiena.com.

LIMITATION OF WARRANTY AND LIABILITY

NOTICE: READ THIS LIMITATION OF WARRANTY AND LIABILITY BEFORE USING THE BAX® SYSTEM EQUIPMENT, ASSAYS, AND/OR MEDIA ("BAX® SYSTEM"). If the terms are not acceptable, notify Hygiena immediately and arrangements will be made for return of the unused Equipment, assays, and/or media to Hygiena and for the refund of the purchase price, less shipping costs. USE OF BAX® SYSTEM EQUIPMENT, ASSAYS AND/OR MEDIA CONSTITUTES AN ACCEPTANCE OF ALL TERMS AND CONDITIONS OF THIS LIMITATION OF WARRANTY AND LIABILITY. Any additional or different terms in Buyer's purchase form(s) are material alterations and hereby rejected.

1. BAX® System Equipment should only be used with BAX® System assays.

2. When used with BAX® System assays, BAX® System Equipment is warranted be free of defects in materials, workmanship and design that may appear under normal and proper use within twelve (12) months from the installation date to the first end user. BAX® System assays are warranted to conform to the assay description under the conditions of use specified in the user documentation to the expiration date stamped on the label. BAX® System media is warranted to meet standard specifications in effect on the date of shipment. Hygiena MAKES NO OTHER WARRANTY, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING. User assumes all risk and liability resulting from use of the BAX® System Equipment, assays and media, whether used singly or in combination with other products.

3. BAX® Software: Hygiena warrants that for a period of 60 days from the date of first date of use by the Customer/end user, BAX® software media will be free from defect in materials and workmanship and that the BAX® software will substantially perform in accordance with the accompanying BAX® software documentation. EXCEPT FOR THE EXPRESS WARRANTY ABOVE, HYGIENA MAKES NO OTHER WARRANTY, EITHER EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY AGAINST INFRINGEMENT, ANY WARRANTY OF MERCHANTABILITY OR OF FITNESS FOR A PARTICULAR PURPOSE OR THOSE ARISING BY LAW, STATUTE, USAGE OF TRADE, OR COURSE OF DEALING. User assumes all risk and liability resulting from use of the BAX® software, whether used singly or in combination with other products.

4. The accuracy of the BAX® System can be affected by factors over which Hygiena has no control, including, without limitation, the use of the Equipment, assays and/or media in a manner that is contrary to the conditions of use, the procedures or the instructions specified by Hygiena. Because of the large number of factors over which Hygiena has no control, Hygiena makes no promise or guarantee of the accuracy of or results obtained from the use of the BAX® System. In particular, Hygiena disclaims any warranty or liability and assumes no responsibility whatever for the failure of the BAX® System due, in whole or in part, to user's failure to: (a) properly maintain Equipment, (b) maintain specified operating or storage conditions, (c) follow the specified instructions, or (d) use the proper microbiological techniques consistent with the standard of care accepted in the industry for the proper collection, storage, handling and preparation of the sample.

5. Externally caused failures, such as improper sample preparation, improper storage or loading of reagents, electrical outages, or out-of-specification environmental conditions are not covered under this warranty. Equipment failures caused by spills, abuse, misuse, negligence, or improper operation are not covered by this warranty. Modifications, service or repairs by parties other than Hygiena-authorized providers are not covered by this warranty and, in fact, void this warranty. Circumstances beyond the reasonable control of Hygiena, including fire, explosions, accidents, flood, labor trouble or shortage, war, act of or authorized by any government, inability to obtain suitable material, Equipment, fuel, power or transportation, or acts of God are not covered under this warranty.

6. The BAX® System is designed to test only for the presence of the target organisms specified in the particular assay. The BAX® System has been tested against many, but not all, strains of the target within the sample types specified in the user documentation. Hygiena, therefore, cannot and does not make any representation or warranty that the BAX® System is capable of detecting every organism in the target genus, serotype, or species in any sample source. Accordingly, the BAX® System should not be used as the sole test for the release of user's products, nor should it be used as the sole basis for determining the safety of user's products.

7. CUSTOMER/USER ASSUMES ALL RISKS IN USING THE BAX® SYSTEM AND HYGIENA OR ITS AFFILIATES, DISTRIBUTORS, ITS LICENSORS OR REPRESENTATIVES SHALL HAVE NO LIABILITY TO CUSTOMER/USER OR TO ANY OTHER PERSON OR ENTITY FOR ANY INDIRECT, INCIDENTAL, SPECIAL, PUNITIVE, EXEMPLARY OR CONSEQUENTIAL DAMAGES WHATSOEVER, INCLUDING, BUT NOT LIMITED TO, LOSS OF REVENUE OR PROFIT, LOST OR DAMAGED DATA OR OTHER COMMERCIAL OR ECONOMIC LOSS EVEN IF CAUSED BY THE NEGLIGENCE OF HYGIENA OR ITS REPRESENTATIVES AND/OR IF HYGIENA HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES, AND/OR IF THEY ARE FORESEEABLE.

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