# Technical Bulletin: Sample Verification for Evaluating Non-Validated Sample Types with Real-Time PCR Assay for *Salmonella*

Before commercialization, each BAX® System PCR assay is internally validated for use with a variety of sample types based on the most common market needs. Most assays are also validated through third party organizations, such as AOAC or AFNOR Certification, for the sample types listed in the BAX® System User Guide. Many sample types use standard enrichment protocols, though some sample types have been validated using alternative media or enrichment protocol modifications.

## Introduction

If your sample type has not been previously validated by or through a third-party organization for use with the BAX® System method, you should perform an internal verification study to determine the appropriate enrichment media, incubation times and incubation temperatures for your sample type. Detailed information about evaluating microbiological methods for use in testing laboratories is available in the memorandum “Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods”, published by the U.S. Food and Drug Administration (FDA) Foods Program Science and Research Steering Committee. You can also perform verification studies according to your laboratory’s standard practices.

For your convenience, a sample verification procedure is provided below which may be used as a guideline for verifying your sample types for use with the BAX® System Real-Time PCR Assay for *Salmonella*.

***Note: To follow the BAX® System method as approved by AOAC or AFNOR Certification, the validated enrichment protocols described in the most current BAX® System User Guide and the kit package insert must be followed.***

## Equipment, Reagents and Supplies

- BAX® System Real-Time PCR Assay for *Salmonella* (KIT2006)
- BAX® System Q7 instrument, equipment and supplies
- Stomacher bags and filtered stomacher bags
- Appropriate enrichment media (see BAX® System User Guide)
- Maximum Recovery Diluent or an equivalent low-nutrient medium (i.e., Peptone Saline Broth, Brain Heart Infusion Broth or Tryptic Soy Broth)
- Nutrient-based agar (i.e., Plate Count Agar or Tryptone Soy Agar)
- Brain Heart Infusion (BHI) broth
- Disposable loops

## Preparing Sample Inoculum

1. To prepare an overnight culture, suspend an isolated colony of *Salmonella* into 8-10 mL of BHI broth. Incubate at 37°C for 18-22 hours. After incubation, the overnight culture is assumed to contain a cell concentration of approximately 109 cfu/mL.

2. Serially dilute the overnight culture 1:10 in Maximum Recovery Diluent or equivalent low-nutrient medium to a concentration of 10 cfu/mL. Keep all dilutions on ice to inhibit further growth.

3. Enumerate the overnight cultures to determine the initial concentrations and actual spike levels.

a. Plate 100 µL of the 100 cfu/mL dilution in triplicate onto a nutrient based agar.

b. Plate 100 µL of the 1,000 cfu/mL dilution in triplicate onto a nutrient based agar.

c. Incubate all plates at 37°C for 18-24 hours.

4. Count the number of colony forming units (cfu) on each plate to determine the total cfu/mL enumeration according to the following formula:

$$N = \frac{\sum C}{[(1 \times n1) + (0.1 \times n2)] \times (d) \times (v)}$$

N = Number of colonies per mL in overnight culture

∑C = Sum of all colonies on all plates counted n1

= Number of plates in first dilution counted

n2 = Number of plates in second dilution counted

d = Dilution from which the first counts were obtained

v= volume plated

In the example below, three plates each were counted from two dilutions

| **Dilution** | **$10^{-6}$ (1,000 cfu/mL)** | **$10^{-7}$ (100 cfu/mL)** |
|---|---|---|
| **Colony Count** | **115, 102, 109** | **11, 14, 8** |

$$N = \frac{115 + 102 + 109 + 11 + 14 + 8}{[(1 \times 3) + (0.1 \times 3)] \times (10^{-6}) \times (0.1)}$$

= 359/0.00000033

= 1.08 x 109 cfu/mL

## Spiking Samples

1. Divide your sample type into the appropriate number of analytical-size portions (see the BAX® System User Guide for reference). For most sample types, a minimum of 12 portions per sample type is recommended.

2. Use the diluted overnight cultures to create high-spike, medium-spike and low-spike samples.

a. To create high-spike samples, inoculate 3 test portions with 100 µL of the 1,000 cfu/mL dilution (about 100 cfu/sample).

b. To create medium-spike samples, inoculate 3 test portions with 100 µL of the 100 cfu/mL dilution (about 10 cfu/sample).

c. To create low-spike samples, inoculate 3 test portions with 100 µL of the 10 cfu/mL dilution (about 1 cfu/sample).

d. Leave the remaining 3 samples unspiked to serve as negative controls.

3. Let samples sit to stress the spiked inoculum and allow it to equilibrate within the test portion.

a. For most refrigerated foods, store test portions at 4°C for 48-72 hours before enrichment.

b. For non-refrigerated foods, store test portions at room temperature for at least two weeks.

c. For frozen foods, store test portions at -20°C for at least two weeks.

## Enriching and Processing Samples

1. Add the appropriate amount of desired enrichment media to each sample. For most sample types, a 1:10 ratio is recommended (e.g., 25 g sample in 225 mL enrichment media).

2. Stomach samples with media for 2 minutes at 200 rpm or homogenize as appropriate.

3. Incubate samples according to the appropriate enrichment protocol for your sample type. For recommendations, see the appropriate standard reference method or the BAX® System User Guide. You can also contact Diagnostics Support for suggested enrichment protocols.

4. Follow the protocol described in the BAX® System User Guide to prepare equipment, perform sample lysis, hydrate PCR tablets and run a full process for the BAX® System Real- Time PCR Assay for *Salmonella*.

## Confirming Results

Perform the appropriate reference culture method to confirm that BAX® System results are accurate for your sample type.

- To confirm results with the ISO method, refer to ISO 6579:2002 or ISO/FDIS 6579:2015 – Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Salmonella* spp.
- To confirm results with the FDA-BAM method, refer to the Bacteriological Analytical Manual (BAM) Chapter 5 – *Salmonella*.
- To confirm results with the USDA-FSIS method, see the Microbiology Laboratory Guidebook (MLG) Chapter 4 – Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges.