AOAC Official Method 2013.02
Salmonella Species in a Variety of Foods and Environmental Surfaces
BAX® System Real-Time PCR Assay for Salmonella
First Action 2013

(Applicable to the detection of Salmonella in a variety of foods, including raw ground beef (25 and 375 g), ground beef with soy (25 and 325 g), beef trim (25 and 325 g), frankfurters (325 g), shrimp (25 g), ground turkey (25 g), chicken wings (25 g), poultry rinse (30 mL), whole powdered (dried) eggs (25 g), shell eggs (1000 mL), fresh bagged lettuce (25 g), frozen peas (25 g), orange juice (pasteurized; 25 mL), cream cheese (25 g), nonfat dry milk (25 g), ice cream (25 g), peanut butter (25 g), cocoa (25 g), white pepper (25 g), milk-based infant formula (25 mL), and dry pet food (375 g), and on stainless steel, ceramic tile, and plastic surfaces.)

See Table 2013.02 for a summary of results of the collaborative study. See Appendix 4, Tables 1–6 for detailed results of the collaborative study [J. AOAC Int. 97, 868(2014)].

Caution: Kits.—The reagents used in the BAX System should pose no hazards when used as directed. Dispose of lysate, PCR mixture, and other waste according to your site practices.

Cycler/detector.—Only qualified laboratory personnel should operate the cycler/detector. Do not attempt to repair the instrument. Live power may still be available inside the unit even when a fuse has blown or been removed. Refer to the BAX System User Guide for maintenance procedures when cleaning the unit or changing a fuse. The heating block can become hot enough during normal operation to cause burns or cause liquids to boil. Wear safety glasses or other eye protection at all times during operation.

Enrichment broths.—All enrichment broths may contain varying pathogens whether they contain Salmonella or not and thus should be sterilized and disposed of using proper procedures following any culture-based confirmatory steps.

Reference cultures.—When handling reference Salmonella cultures, always follow appropriate biosafety containment procedures as provided by your standard laboratory site practices, Centers for Disease Control and Prevention (CDC), or Canadian Pathogen Safety Data Sheets and Risk Assessment.

A. Principle

The DuPont™ BAX System uses the polymerase chain reaction (PCR) to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. The fragment is a genetic sequence that is unique to the genus Salmonella, thus providing a highly reliable indicator that the organism is present. The BAX System simplifies the PCR process by combining the requisite primers, polymerase, and nucleotides into a stable, dry, manufactured tablet already packaged inside the PCR tubes. After amplification, these tubes remain sealed for the detection phase, thus significantly reducing the potential for contamination with one or more molecules of amplified PCR product.

This automated BAX System method uses fluorescent detection to analyze PCR product. One PCR primer for each target (one Salmonella-specific target and an internal control) contains a fluorescent dye (two different dyes, one for each target) as a constituent of the primer as well as a quencher (the unimolecular combination of a primer, fluorescent dye, and quencher constitute a Scorpion™ Probe). When incorporated into a PCR product, the dye and quencher are spatially separated, which causes an increase in emission signal. The BAX System measures the magnitude and characteristics of fluorescent signal change. An analysis by the BAX System software algorithm then evaluates that data to determine a positive or negative result which is displayed as described below.

B. Apparatus and Reagents

Items (a)–(h) are part of the DuPont BAX System Start-Up Package available from DuPont Nutrition & Health (Wilmington, DE, USA; www.fooddiagnostics.dupont.com).

Items (i)–(l) are part of the DuPont BAX System Real-Time PCR Assay for Salmonella from DuPont Nutrition & Health (Cat. No. D14306040).

(a) DuPont BAX System Q7 cycler/detector with computer workstation.

(b) Dupont BAX System application software.

(c) Cluster tubes with caps and racks.—For lysis.

(d) Capping/decapping tools.—For removing and sealing cluster tube caps and PCR tube caps without jarring the contents.

(e) Heating and cooling blocks with inserts.—For maintaining lysis tubes at 37 ± 2, 95 ± 2, and 4°C. [Note: The DuPont Thermal Block (Cat. No. D14614252) may also be used to maintain appropriate temperatures for lysis tubes.]

(f) Pipets.—For transferring reagents; two adjustable mechanical pipets covering 20–200 and 5–50 µL; one repeating pipet; and one multichannel pipet covering eight channels and 550 µL. Pipets should be calibrated to deliver required volumes within 10%.

(g) Pipet tips with barriers.—0.5–250 µL, 0.5–100 µL extended barrier; 5 mL repeater pipet tips.

(h) PCR tube holders.—For transferring a rack of tubes from the cooling block to the cycler/detector.

(i) PCR tubes with tablets.

(j) Flat optical caps for PCR tubes.

(k) Lysis buffer.

(l) Protease.

(m) Incubators.—For maintaining media at 35 ± 1 and 39–42°C.

(n) Stomacher.—Seward model 400 or equivalent for mixing the sponge sample with enrichment media.

(o) Appropriate confirmatory media for culture confirmation.—Rappaport-Vassiliadis Soya Peptone (RVS), Selenite Cystine (SC), tetrahionate-Hajna (TT-Hajna) and tetrahionate (TT) broths, Xylose Lysine Deoxycholate (XLD), Xylose Lysine Tergitol 4 (XLT4), Hektoen Enteric (HE), Brilliant Green Sulfide (BGS), and Bismuth Sulfit (BS) agars.

C. Media

(a) BAX System MP media.—DuPont Cat. No. D12404925 (bulk powder) or D12745725 (StatMedia™ soluble packets).

(b) Brain Heart Infusion (BHI) broth.—Oxoid Cat. No. CM 1032 or equivalent.

(c) Buffered Peptone Water (BPW).—Oxoid Cat. No. CM 0509 or equivalent.
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<th>Frankfurters (325 g)</th>
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</table>

<sup>a</sup> Results include 95% confidence intervals.

<sup>b</sup> Repeatability standard deviation.

<sup>c</sup> Among-laboratory standard deviation.

<sup>d</sup> Reproducibility standard deviation.
(d) mTSB+n.—Oxoid Cat. No. CM0989B or equivalent plus 2 mg/L novobiocin. Autoclave at 121°C for 15 min before addition of filter-sterilized novobiocin.

(e) mTSB+caa+n.—Oxoid Cat. No. CM0989B or equivalent plus 10 g/L casamino acids (casein acid hydrolysate) and 8 mg/L novobiocin. Autoclave at 121°C for 15 min before addition of filter-sterilized novobiocin.

(f) Lactose broth (LB).—Oxoid Cat. No. CM0137 or equivalent.

(g) Brilliant green water.—Prepare brilliant green water by adding 2 mL 1% brilliant green dye solution, C(j), per 1000 mL sterile distilled water. Let container stand undisturbed for 60 ± 5 min. Incubate loosely capped container, without mixing or pH adjustment, at 35°C for 24 ± 2 h.

(h) Reconstituted nonfat dry milk.—Suspend 100 g dehydrated nonfat dry milk in 1 L distilled water. Swirl until dissolved. Autoclave at 121°C for 15 min.

(i) Universal preenrichment broth.—Add 5 g tryptone, 5 g proteose peptone, 15 g potassium phosphate, 7 g sodium phosphate, 5 g sodium chloride, 0.5 g dextrose, 0.25 g magnesium sulfate, 0.1 g ferric ammonium citrate, and 0.2 g sodium pyruvate to 1 L distilled water. Heat ingredients with gentle agitation to dissolve, dispense, and autoclave at 121°C for 15 min. Final pH should be 6.3 ± 0.2.

(j) 1% Aqueous brilliant green dye solution.—Dissolve 1 g dye in sterile water. Dilute to 100 mL.

(k) Tryptic soy broth (TSB).—Suspend 17 g tryptose, 3 g phytone, 5 g sodium chloride, 2.5 g potassium phosphate dibasic, and 2.5 g glucose in 1 L distilled water. Heat gently to dissolve, dispense into containers, and then autoclave 15 min at 121°C. Final pH is 7.3 ± 0.2.

D. Sample Enrichment

(a) Ground beef, ground beef with soy, beef trim (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) BPW, C(e). Incubate, B(m), at 35°C for 20–24 h.

(b) Ground beef (375 g).—Weigh 375 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 1500 mL prewarmed (45°C) mTSB+n, C(d). Incubate, B(m), at 39–42°C for 22–26 h.

(c) Ground beef with soy (325 g).—Weigh 325 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 975 mL prewarmed (35°C) mTSB+caa+n, C(e). Incubate, B(m), at 35°C for 20–24 h.

(d) Beef trim (325 g).—Weigh 325 g test portion into sterile container. Hand massage to homogenize sample for 2 min with 1500 mL prewarmed (41°C) BAX System MP media, C(a). Incubate, B(m), at 39–42°C for 16–24 h.

(e) Frankfurters (325 g).—Weigh 325 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 1400 mL prewarmed (35°C) BPW, C(e). Add additional BPW to reach a total media volume of 2925 mL. Incubate, B(m), at 35°C for 18–24 h.

(f) Shrimp and peanut butter (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) LB, C(f). Let stand at room temperature for 55–65 min. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

Note: Regrowth is required for peanut butter.

(g) Ground turkey and chicken wings (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) BPW, C(e). Incubate, B(m), at 35°C for 16–24 h.

(h) Poultry rinse (30 mL).—Combine 30 mL BPW rinsate with 30 mL prewarmed (35°C) BPW, C(e), into sterile container. Incubate, B(m), at 35°C for 22–26 h.

(i) Dried eggs (25 g).—Weigh 25 g test portion into sterile container. Add approximately 15 mL prewarmed (35°C) LB, C(f), to sample and stir to smooth. Add three additional aliquots of LB of 10, 10, and 190 mL (total media volume 225 mL), stirring after each addition. Let stand at room temperature for 55–65 min. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

(j) Dried eggs, ice cream, and peanut butter (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) BPW, C(e). Incubate, B(m), at 35°C for 22–26 h.

Note: Regrowth is required for peanut butter.

(k) Shell eggs (approximately 1000 g).—Combine 20 eggs into sterile container with 2000 mL prewarmed (42°C) BAX System MP media, C(a). Incubate, B(m), at 42°C for 48 h.

(l) Fresh bagged lettuce (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) LB, C(f). Let stand at room temperature for 55–65 min. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

(m) Dried eggs (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) BAX System MP media, C(a). Incubate, B(m), at 35°C for 22–26 h.

(n) Cream cheese (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) BAX System MP media, C(a). Incubate, B(m), at 35°C for 12–24 h.

(o) Fresh bagged lettuce (25 g).—Weigh 25 g test portion into sterile container. Add 225 mL prewarmed (35°C) LB, C(f), and swirl 25 times clockwise and 25 times counterclockwise. Let stand at room temperature for 55–65 min. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

(p) Fresh bagged lettuce (25 g).—Weigh 25 g test portion into sterile container. Add 225 mL prewarmed (35°C) BAX System MP media, C(a), and swirl 25 times clockwise and 25 times counterclockwise. Incubate, B(m), at 35°C for 10–24 h.

(q) Ice cream (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) Brilliant green water, C(g). Incubate, B(m), at 35°C for 22–26 h.

(r) Orange juice (25 mL).—Weigh 25 g test portion into 225 mL prewarmed (35°C) universal preenrichment broth, C(i), and swirl thoroughly. Let stand at room temperature for 55–65 min. Do not mix or adjust pH. Incubate, B(m), at 35°C for 22–26 h.

Note: Regrowth is required for this sample type.

(s) Orange juice (25 mL).—Weigh 25 g test portion into 225 mL prewarmed (41°C) BAX System MP media, C(a), and swirl thoroughly. Incubate, B(m), at 39–42°C for 22–26 h.

Note: Regrowth is required for this sample type.

(t) Nonfat dry milk (25 g).—Pour 25 g sample slowly over the surface of 225 mL prewarmed (35°C) brilliant green water, C(g).
Let stand at room temperature for 55–65 min. Do not mix or adjust pH. Incubate, B(m), at 35°C for 22–26 h.

Note: Regrowth is required for this sample type.

(u) Stainless steel, ceramic tile, and plastic.—Add 225 mL prewarmed (35°C) LB, C(f), to environmental sponge in sample bag and swirl thoroughly. Let stand at room temperature for 55–65 min. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

(v) Stainless steel, ceramic tile, and plastic.—Add 225 mL prewarmed (35°C) BPW, C(e), to environmental sponge in sample bag and swirl thoroughly. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 18–24 h.

(w) Cocoa (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL reconstituted nonfat dry milk, C(h). Let stand at room temperature for 55–65 min, and then swirl thoroughly to mix. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Add 0.45 mL 1% aqueous brilliant green dye solution, C(j), and mix well. Incubate, B(m), at 35°C for 22–26 h. Transfer 10 mL enrichment to 500 µL BHI broth, C(b), before processing. No additional incubation is required.

(x) White pepper (25 g).—Weigh 25 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with 225 mL prewarmed (35°C) TSB, C(k). Let stand at room temperature for 55–65 min. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

(y) Dry pet food (375 g).—Weigh 375 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with approximately one-third to one-half of 3375 mL prewarmed (35°C) LB, C(f). Add the remainder of the prewarmed media. Let stand at room temperature for 55–65 min, and then swirl thoroughly to mix. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

Note: Regrowth is required for this sample type.

(z) Dry pet food (375 g).—Weigh 375 g test portion into sterile container. Use a stomacher, B(n), to homogenize sample for 2 min with approximately one-third to one-half of 3375 mL prewarmed (35°C) BPW, C(e). Add the remainder of the prewarmed media. Adjust pH to 6.8 ± 0.2 using 1 N HCl or 1 N NaOH, if necessary. Incubate, B(m), at 35°C for 22–26 h.

Note: Regrowth is required for this sample type.

E. Regrowth

(a) After incubation, transfer 10 µL of the enrichment to 500 µL prewarmed (37°C) BHI broth, C(b). Incubate, B(m), at 37°C for 3 h.

(b) Regrowth is required for orange juice, nonfat dry milk, peanut butter, and dry pet food samples. For cocoa, a dilution without additional incubation is required. For all other matrices, regrowth is either optional or not required.

F. Assay

(a) After enriching the sample, turn on the heating blocks, B(e), and set temperatures to 37 and 95°C. Make sure that the cooling blocks have been refrigerated overnight or otherwise chilled at 2–8°C.

(b) Create a rack file by following prompts in the Rack Wizard, B(b), to enter identifying data on the entire rack and on the individual samples.

(c) Label and arrange cluster tubes, B(c), in the cluster tube rack, according to the rack file.

(d) Prepare the lysis reagent by adding 150 µL protease, B(l), to one 12 mL bottle lysis buffer, B(k). Transfer 200 µL prepared lysis reagent to each of the cluster tubes.

(e) Transfer 5 µL enriched sample to the corresponding cluster tubes. Secure caps with the capping/decapping tool, B(d).

(f) Heat cluster tubes at 37°C for 20 min.

(g) Heat cluster tubes at 95°C for 10 min.

(h) Cool cluster tubes at 2–8°C for at least 5 min.

(i) Warm up the cycler/detector, B(a), by selecting RUN FULL PROCESS from the Operations menu of the application window, B(b).

(j) Place a PCR tube holder, B(h), on the PCR cooling block, B(e). Insert one PCR tube, B(i), per sample into the holder and remove caps with the capping/decapping tool, B(d).

(k) Using a multichannel pipet, B(f), transfer 30 µL of sample lysate to PCR tubes, B(l). Seal with flat optical caps, B(j), with the capping/decapping tool, B(d).

(l) Follow screen prompts, B(b), to load samples into the cycler/detector, B(a), and begin the program. At the completion of the PCR and detection process, follow the screen prompts to remove samples and display results.

G. Assay Results

The results are recorded on the rack display or from a spreadsheet printout of the results (called Detail View). Negative results are indicated by a green circle with (–) symbol, positive results are indicated by a red circle with (+) symbol, and indeterminate results are indicated with a yellow circle with (?) symbol. A yellow circle with a (?) symbol and a red slash indicate a low signal or signal error.

BAX System results are displayed as in Figure 2013.02.

H. Confirmation

Presumptive positive results are confirmed by culture and the biochemical and serological protocols described in the appropriate reference method relevant to the matrix. For meat, poultry, and pasteurized egg products, follow the USDA-FSIS MLG Chapter 4 (http://www.fsis.usda.gov/wps/wcm/connect/700c05fe-06a2-492a-a6e1-3357f7701f52/MLG-4.pdf?MOD=AJPERES). For all other matrices, follow the FDA-BAM Chapter 5 (http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm). Alternatively, matrices may be confirmed as described in the Health Canada Compendium, Vol. 3, Laboratory Procedures for the Microbiological Examination of Foods, Health Canada, Health Products and Food Branch, where appropriate (http://www.hc-sc.gc.ca/fn-an/res-rech/analys-meth/microbio/volume3-eng.php).

Reference: J. AOAC Int. 97, 868(2014)