

Hygiena™ BAX® System PCR Assay for *Salmonella* Dilution and regrowth recommendations for untreated herbs and spices

The AOAC-approved protocol for testing black pepper with the BAX® System *Salmonella* assay specifies a 1:10 dilution in TSB for pre-enrichment, with a 1:50 regrowth dilution in BHI. An internal study investigated the BAX® System performance using 4 dilution levels of BPW for pre-enrichment on 49 untreated herb and spice products, including several types of ground pepper. Results showed that BPW is an effective enrichment medium for most of the food types tested using 1:10 or 1:20 dilutions. Results also showed that more than half of the food types did not require a regrowth step.

Methodology

Equipment and reagents

BAX® System PCR Assay for *Salmonella* (KIT2012)

BAX® System Q7 instrument

BAX® System equipment and supplies

Buffered Peptone Water (BPW)

Brain-Heart Infusion (BHI) broth

Rappaport-Vassiliadis (RV) medium

Tetrathionate (TT) broth

Bismuth Sulfite (BS) agar

Xylose Lysine Deoxycholate (XLD) agar

Hektoen Enteric (HE) agar

Salmonella Dublin strain

Sample preparation and inoculation

For each food type, pairs of 10 g samples were weighed into separate stomacher bags containing BPW and homogenized. Dilution volumes (see Table 1) were determined by reference method recommendations or experience with the matrix. One sample from each pair was left uninoculated as a negative control. The other sample was spiked with a 0.5 mL aliquot of *Salmonella* Dublin that was diluted to 10^2 CFU/mL.

Table 1. Dilution volumes

Dilution	Volume of BPW per 10 g sample
1:10	90 mL
1:20	190 mL
1:50	490 mL
1:1000	999 mL (1 g sample)

Unspiked samples were incubated for 22 hours at 42°C. Spiked samples were incubated for 20 hours at 42°C, then 5 µL was removed for immediate processing with the BAX® System. At the same time, another 10 µL was transferred to 500 µL of BHI and incubated for an additional 3 hours at 42°C before processing with the BAX® System.

Method

BAX® System protocol – Lysis reagent was prepared (150 µL protease + 12 mL lysis buffer) and added in 200 µL aliquots to two sets of cluster tubes. Five-microliter aliquots of enriched/regrown samples were added to the cluster tubes. Tubes were placed in a heating block for 20 minutes at 37°C, then for 10 minutes at 95°C, and cooled for 5 minutes at 2-8°C. PCR tubes were arranged on a cold (2-8°C) block and 50 µL aliquots of lysate were added for processing in the BAX® System instrument.

FDA-BAM reference method protocol – For each spiked sample, 1 mL was added to 10 mL of RV medium and another 1 mL sample was added to 10 mL of TT broth. Samples were vortexed briefly and then incubated for 24 hours at 42°C. After incubation, 10 µL from each secondary enrichment was streaked onto each of three agars (BS, XLD, HE) and incubated for another 24 hours at 35°C.

Confirmation

All samples were processed using the BAX® System and were confirmed using the reference method.

Results

The BAX® System detected *Salmonella* in all 49 spiked samples that were pre-enriched in BPW. Twenty-one of the spiked samples were positive using 1:10 or 1:20 dilutions and no regrowth. Steak and roast seasoning required a 1:50 dilution and cinnamon required a 1:1000 dilution (Table 2). The remaining 26 spiked samples were positive at 1:10 or 1:20 dilutions, but required a regrowth step (Table 3). All unspiked controls were negative.

Table 2. Recommended dilutions for food types that did not require a regrowth step

Sample type	Recommended enrichment dilution	Results		
		BAX	Confirmed	FDA-BAM
Cayenne pepper, whole organic	1:10	+	+	+
Chicken gravy mix	1:10	+	+	+
Onion, sliced	1:10	+	+	+
Pancake mix	1:10	+	+	+
Pepper, Tellicherry, whole	1:10	+	+	+
Pepper, white, ground	1:10	+	+	+
Poppy seeds	1:10	+	+	+
Pork gravy mix	1:10	+	+	+
Sesame seeds	1:10	+	+	+
Bing cherries, dried	1:20	+	+	+
Chili pepper, ancho-type	1:20	+	+	+
Cumin, ground	1:20	+	+	+
Flavor base	1:20	+	+	+
Garlic, roasted granulated	1:20	+	+	+
Ginger, ground organic	1:20	+	+	+
Parsley, powdered	1:20	+	+	+
Parsley flakes, organic	1:20	+	+	+
Pepper, red, crushed	1:20	+	+	+
Pepper, red, ground	1:20	+	+	+
Tomato, powdered with anticaking agent	1:20	+	+	+
Walnut pieces	1:20	+	+	+
Steak & roast seasoning	1:50	+	+	+
Cinnamon, ground	1:1000	+	+	+

Table 3. Recommended dilutions for food types that require a regrowth step

Sample type	Recommended enrichment dilution	Results		
		BAX*	Confirmed*	FDA-BAM*
Apricots, dried	1:10	+	+	+
Garlic, minced	1:10	+	+	+
Macadamia nut, whole	1:10	+	+	+
Pistachio nut granules	1:10	+	+	+
Star anise, whole	1:10	+	+	+
Basil, ground	1:20	+	+	+
Basil, whole	1:20	+	+	+
Basil, whole organic	1:20	+	+	+
Cajun-style blackened fish seasoning	1:20	+	+	+
Celery stalk and leaf, powdered	1:20	+	+	+
Fajita seasoning	1:20	+	+	+
Garlic, granulated Chinese	1:20	+	+	+
Garlic, powdered	1:20	+	+	+
Greek seasoning without MSG	1:20	+	+	+
Green & red bell pepper, dehydrated and chopped	1:20	+	+	+
Gumbo filé	1:20	+	+	+
Herb seasoning	1:20	+	+	+
Lemon & herb seasoning	1:20	+	+	+
Marjoram, whole	1:20	+	+	+
Mustard flour	1:20	+	+	+
Peppercorns, whole pink	1:20	+	+	+
Poultry and meat rub	1:20	+	+	+
Poultry seasoning	1:20	+	+	+
Tarragon leaf	1:20	+	+	+
White peppered biscuit gravy	1:20	+	+	+
Yellow rice seasoning	1:20	+	+	+

Conclusions

BPW is an effective enrichment for the herbs and spices tested in this study, with good recovery at 1:10 or 1:20 dilutions for all but two food types. Although the BAX® System detected positives in almost half the samples without a regrowth step, Hygiena™ recommends regrowth for all herbs and spices, when time permits, to overcome potential PCR or microbial growth inhibition. Although not part of the AOAC-approved method, these recommended BPW dilutions offer helpful enrichment alternatives to labs with testing protocols that are challenged by herbs and spices.