

Technical Bulletin: Detection of *Salmonella* from Garlic Powder using the BAX® System PCR Assays



An internal validation study was conducted to measure the method performance of the BAX® System Real-Time and standard PCR assays for *Salmonella* compared to the U. S. Food and Drug Administration’s Bacteriological Analytical Manual (FDA BAM) reference method for the detection of *Salmonella* in 25 g samples of garlic powder. Paired samples tested in this study were inoculated with a low level expected to produce fractional positive results and a high level expected to produce all positive results. Samples were held for 72 hours, enriched and tested. The results were analyzed using the probability of detection (POD), demonstrating equivalent performance between the BAX® System method and the reference method.

Introduction

Garlic is one of the most commonly used ingredients as a flavor enhancement for foods (1). The bioactive compounds in garlic that create its pungent taste also contribute towards its antimicrobial properties. Yet, garlic powder among other spices have been implicated in recalls and outbreaks which were contaminated with *Salmonella*. The recovery and detection of *Salmonella* in spices remain a challenge and requires neutralization to counteract antimicrobial compounds (2).

Sample Preparation and Enrichment

Garlic powder was divided into 25 g test portions and inoculated with *Salmonella* Typhimurium to create 20 low-level samples and 5 high-level samples. Five samples were left uninoculated for negative controls. All samples were held at room temperature for 72 hours to equilibrate the target organism in the matrix.

Samples were then homogenized with 225 mL of TSB + K₂SO₃, held at room temperature for 60 minutes and incubated at 35°C for 24 hours.

See Figure 1 (note: sample enrichments in this study were not pH adjusted since measurements were within the correct range of 6.8 ± 0.2)

Method

BAX® System Method

All samples were processed following the procedures for Real-Time *Salmonella* (KIT2006), *Salmonella* 2 (KIT2011) and X5 *Salmonella* (KIT2025) described in the BAX® System Q7 and X5 Users Guide.

Reference Method

All samples were culture confirmed regardless of BAX® System results following the FDA BAM Chapter 5 for *Salmonella*.

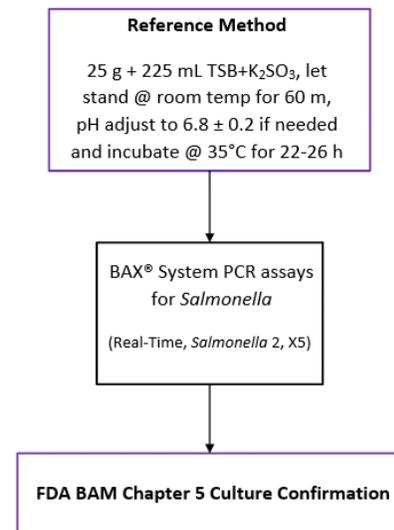


Figure 1. Paired study using the FDA BAM reference enrichment method and BAX® System method for garlic powder.

Results

All three BAX® System PCR assays (Real-Time *Salmonella*, *Salmonella* 2, and X5 *Salmonella*) returned positive results for 7/20 low spiked samples and 4/5 high spiked samples at 24 hours, with and without a 3-hour BHI regrowth. When compared to culture, all BAX® System results were identical.

To compare the method performance, the BAX[®] System presumptive and confirmed results were analyzed using the probability of detection (POD). No significant difference was determined since the 95% confidence interval includes zero (Table 1).

Table 1. BAX [®] System Presumptive vs. Confirmed Results										
Sample Type	CFU/Test Portion	N	BAX [®] System Presumptive			BAX [®] System Confirmed			dPOD _{CP}	95% CI
			X	POD _{CP}	95% CI	X	POD _{CC}	95% CI		
Garlic Powder (25 g)	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
	69.1	20	7	0.35	0.18, 0.56	7	0.35	0.18, 0.56	0.00	-0.28, 0.28
	691.2	5	4	0.80	0.37, 0.96	4	0.80	0.37, 0.96	0.00	-0.45, 0.45

N = Number of test portions

X = Number of positive test portions

POD_{CP} = BAX[®] System method presumptive positive results divided by the total number of test portions

POD_{CC} = BAX[®] System method confirmed positive results divided by the total number of test portions

dPOD_{CP} = Difference between the BAX[®] System method presumptive result and BAX[®] System method confirmed result
POD values

95% CI = If the confidence interval of dPOD does not contain zero, then the difference is statistically significant at the 5% level

Conclusions

Overall, the BAX[®] System PCR assays can accurately and reliably detect *Salmonella* from garlic powder equivalent to culture using the following enrichment protocol:

- Homogenize 25 g sample with 225 mL of TSB + K₂SO₃, let stand at room temperature for 60 minutes, pH adjust to 6.8 ± 0.2 if necessary and incubate at 35°C for 22-26 hours.

References

1. Sallam K. I., Ishioroshi, M., and Samejima, K. December 2004. Antimicrobial effects of garlic in chicken sausage. LWT – Food Science and Technology. 37(8): 849–855.
2. Barrere, V., Tompkins, E., Armstrong, M., Bird, P., Bastin, B., Goodbridge, L. March 2, 2020. Optimization of Salmonella detection in garlic, onion, cinnamon, red chili pepper powders and green tea. Int J Food Microbiology. 316:108440.