Technical Bulletin: Detection of *Salmonella* and *Listeria* from Whey Protein Concentrate 80 Using the BAX® System Real-Time PCR Assays

An unpaired study was conducted by an independent laboratory to compare the performance of the BAX® System Real-Time PCR Assay for *Salmonella* and Real-Time PCR Assay for Genus *Listeria* against the United States Food and Drug Administration's (FDA) reference methods to detect each target organism in whey protein concentrate 80 (WPC 80). Samples tested in this study were artificially inoculated at levels expected to produce low (0.2-2 cfu/test portion) or high (5 cfu/test portion) spike levels after a 2 week equilibration at room temperature. The results obtained were analyzed using the probability of detection (POD). For *Salmonella*, the BAX® System method with a BHI regrowth demonstrated superior performance to the reference method at the low inoculation level. For *Listeria*, the BAX® System method demonstrated equivalent performance to the reference method.

Introduction

Whey is a natural byproduct of milk that is used as a value-added ingredient for different food sectors including sports food, bakery, seasonings and flavors (*1*). To produce whey, dairy farmers and processors must abide by strict sanitary and quality standards to prevent contamination with pathogenic bacteria (*2*). Therefore, reliable screening methods capable of detecting pathogens must be evaluated in finished product.

Sample Preparation and Enrichment

Salmonella Typhimurium ATCC 13311 and *Listeria ivanovii* ATCC 19119 were used to inoculate samples of WPC 80 independently. For each target organism, WPC 80 was dry inoculated in bulk to create a low fractional spike level or a high spike level. Each master sample was thoroughly mixed and stored at room temperature for 2 weeks. The master samples were then separated into 25 g test portions to create 20 low-spike and 5 high spike samples per method. An additional 5 samples per method were left uninoculated to serve as negative controls.

For the BAX® System Real-Time *Salmonella* method, 375 g test portions were analyzed by combining a 25 g sample of inoculated product with 350 g of uninoculated product. Samples were homogenized with 3.375 L pre-warmed (35°C) Buffered peptone water (BPW) and incubated at 35°C for 22-26 hours. Samples were tested by the BAX® System method directly from the primary enrichment and after a BHI regrowth. The regrowth was performed by transferring 10 µL of the primary enrichment to 500 µL pre-warmed (37°C) BHI broth and incubating at 37°C for 3 hours before proceeding to the lysis procedure described below.

For the BAX® System Real-Time Genus *Listeria* method, 50 g test portions were analyzed by combining a 25 g sample of inoculated product with 25 g of uninoculated product. Samples were homogenized with 450 mL pre-warmed (20-35°C) 24 LEB Complete media and incubated at 35°C for 26-48 hours. Samples were tested by the BAX® System method at 26 and 48 hours.

For the FDA BAM reference methods, 25 g samples were analyzed for both *Salmonella*

and *Listeria*. *Salmonella* samples were homogenized with 225 mL of Lactose broth (LB) and incubated at 35°C for 22-26 hours. *Listeria* samples were homogenized with 225 mL of BLEB containing pyruvate and incubated at 30°C for 4 hours. After 4 hours, solutions of three selective agents (acriflavin, cycloheximide and nalidixic acid) were added to the enrichment, mixed and incubated at 30°C for an additional 44 hours.

Method

BAX® System Method – For *Salmonella* samples, 5 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease to one 12 mL bottle of lysis buffer) in cluster tubes. Lysis was performed by heating tubes for 20 minutes at 37°C and 10 minutes at 95°C, and then cooling tubes at 4°C. Real-Time *Salmonella* PCR tubes were hydrated with 30 µL of lysate and held for 10 minutes. All PCR tubes were then loaded into the BAX® System Q7 instrument, and a full process was run according to the procedure described in the BAX® System User Guide.

For *Listeria* samples, 5 µL of enrichment was added to 200 µL prepared lysis reagent (150 µL of protease and 200 µL of Lysing Agent 2 to one 12 mL bottle of lysis buffer) in cluster tubes. Lysis was performed by heating tubes for 30 minutes at 55°C and 10 minutes at 95°C, and then cooling tubes at 4°C. Real-Time Genus *Listeria* PCR tubes were hydrated with 30 µL of lysate. All PCR tubes were loaded into the BAX® System Q7 instrument, and a full process was run according to the procedure described in the BAX® System User Guide.

Reference Method – Each sample was culture confirmed regardless of presumptive BAX® System results following the FDA BAM Chapter 5 for *Salmonella* or FDA BAM Chapter 10 for *Listeria monocytogenes*.

Results and Discussion

Statistical analysis using the probability of detection (POD) and the difference in POD (dPOD) values were calculated with 95% confidence intervals to compare the results between the BAX® System method presumptive result and BAX® System method confirmed results (Table 1) and between the BAX® System method and reference method (Table 2).

Table 1. BAX® System Presumptive vs. Confimed Results

Sample Type	Test Method	Target Organism	MPN/25 g	Test Portions	BAX® System Presumptive			BAX® System Confirmed			$dPOD_{CP}$	95% CI
					X	POD_{CP}	95% CI	X	POD_{CC}	95% CI		
WPC 80 (375 g)	BAX® System 24 h	*Salmonella* Typhimurium ATCC 13311	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.10	20	2	0.10	0.03, 0.30	11	0.55	0.34, 0.74	-0.45	-0.65, -0.16
			1.91	5	0	0.00	0.00, 0.45	4	0.80	0.37, 0.96	-0.80	-0.96, -0.37
	BAX® System 24 h + BHI regrowth	*Salmonella* Typhimurium ATCC 13311	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.10	20	11	0.55	0.34, 0.74	11	0.55	0.34, 0.74	0.00	-0.28, 0.28
			1.91	5	4	0.80	0.37, 0.96	4	0.80	0.37, 0.96	0.00	-0.45, 0.45
WPC 80 (50 g)	BAX® System 26 h	*Listeria ivanovii* ATCC 19119	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.25	20	6	0.30	0.15, 0.52	8	0.40	0.22, 0.61	-0.10	-0.36, 0.18
			2.43	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45
	BAX® System 48 h	*Listeria ivanovii* ATCC 19119	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.25	20	8	0.40	0.22, 0.61	8	0.40	0.22, 0.61	0.00	-0.28, 0.28
			2.43	5	5	1.00	0.57, 1.00	5	1.00	0.57, 1.00	0.00	-0.45, 0.45

MPN/25 g = Most Probable Number is based on the POD of reference method test portions

X = Number of positive test portions

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

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

$dPOD_{CP}$ = Difference between the BAX® method presumptive result and BAX® 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

Table 2. BAX® System Results vs. Reference Method Results

Sample Type	Test Method Comparison	Target Organism	MPN/25 g	Test Portions	BAX® System Method			Reference Method			$dPOD_C$	95% CI
					X	POD_C	95% CI	X	POD_R	95% CI		
WPC 80	BAX® System 24 h / Reference	*Salmonella* Typhimurium ATCC 13311	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.10	20	2	0.10	0.03, 0.30	2	0.10	0.03, 0.30	0.00	-0.21, 0.21
			1.91	5	0	0.00	0.00, 0.45	5	1.00	0.57, 1.00	-1.00	-1.00, -0.57
	BAX® System 24 h + BHI regrowth / Reference	*Salmonella* Typhimurium ATCC 13311	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.10	20	11	0.55	0.34, 0.74	2	0.10	0.03, 0.30	0.45	0.16, 0.65
			1.91	5	4	0.80	0.37, 0.96	5	1.00	0.57, 1.00	-0.20	-0.62, 0.26
	BAX® System 26 h / Reference	*Listeria ivanovii* ATCC 19119	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.25	20	6	0.30	0.15, 0.52	4	0.20	0.08, 0.42	0.10	-0.16, 0.35
			2.43	5	5	1.00	0.57, 1.00	4	0.80	0.37, 0.96	0.2	-0.26, 0.62
	BAX® System 48 h / Reference	*Listeria ivanovii* ATCC 19119	Control	5	0	0.00	0.00, 0.45	0	0.00	0.00, 0.45	0.00	-0.45, 0.45
			0.25	20	8	0.40	0.22, 0.61	4	0.20	0.08, 0.42	0.2	-0.08, 0.44
			2.43	5	5	1.00	0.57, 1.00	4	0.80	0.37, 0.96	0.2	-0.26, 0.62

MPN/25 g = Most Probable Number is based on the POD of reference method test portions

X = Number of positive test portions

POD_C = Confirmed BAX® method positive results divided by the total number of test portions

POD_R = Confirmed reference method positive results divided by the total number of test portions

$dPOD_C$ = Difference between the BAX® method and reference method POD values

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

For *Salmonella* samples enriched in BPW, the BAX® System method returned positive results for 2/20 low spiked and 0/5 high spiked samples without using a BHI regrowth. When a BHI regrowth is used, the BAX® System method returned positive results for 11/20 low spiked samples and 4/5 high spiked samples identical to culture. The corresponding samples enriched using the FDA BAM method returned culture positive results for 2/20 low spiked samples and all 5 high spiked samples. The statistical analyses between the BAX® System presumptive and confirmed results demonstrates a significant difference for samples tested without using a BHI regrowth indicating a BHI regrowth is necessary. The statistical analyses between the results of the BAX® System method with a BHI regrowth and the reference method demonstrate a significant difference at the low inoculation level, in which the BAX® System method had a higher proportion of positives.

For *Listeria* samples enriched in 24 LEB Complete media, the BAX® System method returned positive results for 6/20 low spiked samples at 26 hours and 8/20 low spiked samples at 48 hours. All 5 high spiked samples were positive at each time. The 48 hour results were identical to culture. The corresponding samples enriched using the FDA BAM method returned culture positive results for 4/20 low spiked samples and 4/5 high spiked. The statistical analyses between the BAX® System presumptive and confirmed results or the BAX® System method and reference method demonstrates no significant difference in any of these comparisons.

Conclusions

Overall, the results of this study demonstrate the ability of the BAX® System Real-Time PCR Assay for *Salmonella* to accurately detect *Salmonella* species in 375 g samples of WPC 80 after a BHI regrowth with superior

performance to the reference method while the Real-Time PCR Assay for Genus *Listeria* accurately detects *Listeria* species in 50 g samples of WPC 80 equivalent to the reference method using the following enrichment protocols:

- For *Salmonella*, homogenize 375 g samples with 3.375 L pre-warmed (35°C) BPW and incubate at 35°C for 24 hours. A 3 hour BHI regrowth is required.
- For *Listeria*, homogenize 50 g samples with 450 mL pre-warmed (20-35°C) 24 LEB Complete media and incubate at 35°C for 26-48 hours.

References

1. U.S. Dairy Export Council. June 2004. Reference Manual for U.S. Whey and Lactose Products. http://usdec.files.cms-plus.com/PDFs/2008ReferenceManuals/Whey_Lactose_Reference_Manual_Complete2_Optimized.pdf
2. Chandan, R.C. Kilara, A., Shah, N. P., Schmidt, R. H. 2008. Diary Processing & Quality Assurance: Microbiological Considerations Related to Diary Processing. 99-133, 209-2.