# Technical Bulletin: Detection of *Salmonella* and *Listeria* from Lactose Powder 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 reference methods to detect each target organism in lactose powder. Samples tested in this study were artificially inoculated at levels expected to create low (0.2-2 CFU/test portion) and 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 both *Salmonella* and *Listeria,* the BAX® System method demonstrated equivalent performance to the reference method.

## Introduction

Lactose is the predominant carbohydrate found in milk. It is extracted from whey using a process of crystallization. Lactose crystals are then used as a functional and nutritional ingredient in food products such as breads, confectioneries and infant formulas. Specifically, for infant nutrition, lactose provides a useful energy source and increases the bioavailability and absorption of calcium. While these properties help meet the need for growing infants, it also creates a nutritious environment for foodborne pathogens (*1*). To minimize the risk to human health, strict sanitary standards and control measures like pasteurization of milk are followed to maximize product safety. However, in the case that pathogens are not killed, finished product must be analyzed.

## Sample Preparation and Enrichment

*Salmonella* I 4,[5],12:i:- and *Listeria welshimeri* ATCC 35897 were used to inoculate samples of lactose powder independently. For each target organism, lactose powder was dry inoculated in bulk to create a low fractional spike level or a high spike level. Each master sample was thoroughly mixed to achieve equal distribution of the inoculum 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 1500 mL 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, 25 g test portions were analyzed. Samples were homogenized with 225 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 Universal pre-enrichment broth (UPB) 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 (acriflavine, 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 on a chilled (2-8°C) cold block. 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 – All samples were 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 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 | | |
| Lactose Powder (375 g) | BAX® System 24 h with and without a BHI regrowth | *Salmonella* I 4,[5],12:i:- | Control | 5 | 0 | 0.00 | 0.00, 0.45 | 0 | 0.00 | 0.00, 0.45 | 0.00 | -0.45, 0.45 |
| | | | 0.74 | 20 | 10 | 0.50 | 0.30, 0.70 | 10 | 0.50 | 0.30, 0.70 | 0.00 | -0.28, 0.28 |
| | | | 4.92 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | -0.43, 0.43 |
| Lactose Powder (25 g) | BAX® System 26 and 48 h | *Listeria welshimeri* ATCC 35897 | Control | 5 | 0 | 0.00 | 0.00, 0.45 | 0 | 0.00 | 0.00, 0.45 | 0.00 | -0.45, 0.45 |
| | | | 1.3 | 20 | 12 | 0.60 | 0.38, 0.78 | 12 | 0.60 | 0.38, 0.78 | 0.00 | -0.28, 0.28 |
| | | | 4.92 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | -0.43, 0.43 |

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 | | |
| Lactose Powder (375 g) | BAX® System 24 h / Reference | *Salmonella* I 4,[5],12:i:- | Control | 5 | 0 | 0.00 | 0.00, 0.45 | 0 | 0.00 | 0.00, 0.45 | 0.00 | -0.45, 0.45 |
| | | | 0.74 | 20 | 10 | 0.50 | 0.30, 0.70 | 13 | 0.65 | 0.43, 0.82 | -0.15 | -0.41, 0.14 |
| | | | 4.92 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | -0.43, 0.43 |
| Lactose Powder (25 g) | BAX® System 26 and 48 h / Reference | *Listeria welshimeri* ATCC 35897 | Control | 5 | 0 | 0.00 | 0.00, 0.45 | 0 | 0.00 | 0.00, 0.45 | 0.00 | -0.45, 0.45 |
| | | | 1.3 | 20 | 12 | 0.60 | 0.38, 0.78 | 14 | 0.70 | 0.48, 0.85 | -0.10 | -0.36, 0.18 |
| | | | 4.92 | 5 | 5 | 1.00 | 0.57, 1.00 | 5 | 1.00 | 0.57, 1.00 | 0.00 | -0.43, 0.43 |

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 10/20 low spiked and all 5 high spiked samples with and without a BHI regrowth. All results were identical to culture. The corresponding samples enriched using the FDA BAM method returned culture positive results for 13/20 low spiked and all 5 high spiked samples. The statistical analyses between the BAX® System presumptive and confirmed results as well as the BAX® System method and reference method demonstrated no significant difference.

For *Listeria* samples enriched in 24 LEB Complete media, the BAX® System method returned positive results for 12/20 low spiked and all 5 high spiked samples at both 26 and 48 hours. All results were identical to culture. The corresponding samples enriched using the FDA BAM method returned culture positive results for 14/20 low spiked and all 5 high spiked samples. The statistical analyses between the BAX® system presumptive and confirmed results as well as the BAX® System method and reference method demonstrated no significant difference.

## Conclusions

Overall, the results of this study demonstrate the ability of the BAX® System Real-Time PCR Assay for *Salmonella* and Real-Time PCR Assay for Genus *Listeria* to accurately detect *Salmonella* species and *Listeria* species, respectively, in lactose powder equivalent to the reference methods using the following enrichment protocols:

- For *Salmonella*, homogenize 375 g samples with 1500 mL pre-warmed (35°C) BPW and incubate at 35°C for 24 hours.
- For *Listeria*, homogenize 25 g samples with 225 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