

Detecting Listeria in Mozzarella Cheese with DuPont™ BAX® System Real-Time PCR



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Introduction

Although Listeria contamination more commonly occurs in dairy products made using unpasteurized (raw) milk, food products made from pasteurized milk, like semi-soft mozzarella cheese, can also become contaminated under unsanitary conditions. For this reason, reliable microbiological testing methods are needed to prevent Listeria-related illnesses in a variety of dairy products.

The following study was performed as part of the DuPont Nutrition & Health Protect the Base program to validate the BAX® System Real-Time PCR Assay for Genus Listeria for detecting this pathogen in samples of mozzarella cheese made with part-skim milk.



Equipment, Supplies and Reagents

- DuPont BAX System Q7 Startup Package (Cat. No. **10754-678**)
- DuPont BAX System Real-Time PCR Assay for Genus Listeria (Cat. No. **10754-874**)
- Incubators capable of maintaining designated temperatures within $\pm 2^{\circ}\text{C}$ (Cat. No. **89511-428**)
- 24 LEB Complete Media (Cat. No. **10754-926**)
- Brain Heart Infusion Broth (BHI)
- Modified Oxford (MOX) Agar (Cat. No. **89407-326**)

Sample Preparation and Enrichment

To artificially contaminate mozzarella cheese samples, a strain of Listeria monocytogenes was selected from the DuPont Nutrition & Health Culture Collection and grown overnight at 35°C in BHI broth. The culture was then serially diluted in additional BHI broth to a dilution level appropriate for achieving fractional positive results.

Mozzarella cheese made with part-skim milk was divided into 125g analytical test portions. Twenty portions were inoculated with the diluted L. monocytogenes strain

at the fractional level, five portions were inoculated at a level approximately 10x the fractional level, and five portions were left un-inoculated to serve as negative controls. All portions were held at 4°C for 72 hours to cold stress the target organism before analysis.

Each test portion was homogenized with 1125mL pre-warmed (35°C) 24 LEB Complete media and incubated at 35°C . Samples were tested by the BAX® System method at after 22, 24 and 26 hours of enrichment.

BAX System Method

Lysis reagent was prepared by adding $150\mu\text{L}$ of protease and $200\mu\text{L}$ of Lysing Agent 2 to one 12mL bottle of lysis buffer. For each sample, $200\mu\text{L}$ prepared lysis reagent and $5\mu\text{L}$ enriched sample was added to cluster tubes. Lysis was performed by heating tubes for 30 minutes at 55°C followed by 10 minutes at 95°C . Samples were chilled at $2-8^{\circ}\text{C}$ for at least 5 minutes, then $30\mu\text{L}$ sample lysate was transferred to the BAX System PCR tubes. Samples were allowed to sit for 10 minutes, then

processed in the BAX System Q7 instrument according to the procedure described in the BAX System User Guide.

Results and Discussion

Of the 20 fractionally spiked samples tested, the BAX System method returned 10 positive results. All five samples inoculated at 10x the fractional concentration returned positive results with the BAX System method. All negative control samples returned negative results.

All samples, regardless of result, were confirmed by streaking enrichments onto MOX agar plates. Results from the culture confirmation demonstrated 100% correlation with the BAX System results.

Conclusions

The results of this study demonstrate the DuPont BAX System Real-Time PCR Assay for Genus Listeria can accurately and reliably detect Listeria in 125g samples of mozzarella cheese after 22–26 hours of enrichment in 24 LEB Complete media.



| Sample | Inoculation Level (cfu/portion) | BAX System Result | | | Culture Confirmation | |
|--------|---------------------------------|-------------------|---------|---------|----------------------|---------|
| | | 22 hour | 24 hour | 26 hour | 24 hour | 48 hour |
| 1 | 0 | NEG | NEG | NEG | NEG | NEG |
| 2 | 0 | NEG | NEG | NEG | NEG | NEG |
| 3 | 0 | NEG | NEG | NEG | NEG | NEG |
| 4 | 0 | NEG | NEG | NEG | NEG | NEG |
| 5 | 0 | NEG | NEG | NEG | NEG | NEG |
| 6 | 0.9 | POS | POS | POS | POS | POS |
| 7 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 8 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 9 | 0.9 | POS | POS | POS | POS | POS |
| 10 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 11 | 0.9 | POS | POS | POS | POS | POS |
| 12 | 0.9 | POS | POS | POS | POS | POS |
| 13 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 14 | 0.9 | POS | POS | POS | POS | POS |
| 15 | 0.9 | NEG | NEG | NEG | NEG | NEG |

| Sample | Inoculation Level (cfu/portion) | BAX System Result | | | Culture Confirmation | |
|--------|---------------------------------|-------------------|---------|---------|----------------------|---------|
| | | 22 hour | 24 hour | 26 hour | 24 hour | 48 hour |
| 16 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 17 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 18 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 19 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 20 | 0.9 | NEG | NEG | NEG | NEG | NEG |
| 21 | 0.9 | POS | POS | POS | POS | POS |
| 22 | 0.9 | POS | POS | POS | POS | POS |
| 23 | 0.9 | POS | POS | POS | POS | POS |
| 24 | 0.9 | POS | POS | POS | POS | POS |
| 25 | 0.9 | POS | POS | POS | POS | POS |
| 26 | 9 | POS | POS | POS | POS | POS |
| 27 | 9 | POS | POS | POS | POS | POS |
| 28 | 9 | POS | POS | POS | POS | POS |
| 29 | 9 | POS | POS | POS | POS | POS |
| 30 | 9 | POS | POS | POS | POS | POS |

Table 1: BAX System Results in 125g Samples of Mozzarella Cheese

AOAC-RI Approved PCR Tests for Listeria with the BAX® System X5 PCR

The AOAC Research Institute has approved method extensions of Performance Tested Methods™ 030502 and 070202 to include the latest DuPont BAX System X5 PCR Assays for Genus Listeria and Listeria monocytogenes, respectively. These approvals validate the assays as reliable methods for detecting Listeria species in frankfurters, smoked salmon, spinach, cheese, dairy products, seafood, fresh produce, and from environmental surfaces.

As the exclusive US distributor of the DuPont BAX System X5, VWR has all consumables required for rapid, PCR based detection of foodborne pathogens. Go to market with confidence after confirming that your raw ingredients, work surfaces or environments, and finished products are free of harmful organisms.



| Description | Size | Cat. No. | Unit |
|--|----------|-----------|-------------|
| BAX System X5 Start Up Package | — | 10002-882 | Each |
| BAX System X5 PCR Assay for Listeria monocytogenes | 64 Tests | 10002-774 | Each |
| BAX System X5 PCR Assay for Genus Listeria | 64 Tests | 10002-772 | Each |
| Pathogen Testing Supporting Materials | | | |
| FoodChek Actero™ for Listeria | 500 g | 97068-830 | Each |
| Enrichment Broth Components | | | |
| BD Difco™ Demi-Fraser Broth Base | 500 g | 90003-776 | Each |
| Hardy Diagnostics Half Fraser Broth Base, CRITERION™ | 500 g | 89406-644 | Each |
| Hardy Diagnostics Fraser Broth Supplement for Listeria | 100 mL | 89426-214 | Each |
| HiMedia Listeria Enrichment HiVeg Media | 500 g | 61001-412 | Each |
| HiMedia Supplement I, Listeria UVM Selective | 5 Packs | 71005-820 | Pack of 5 |
| HiMedia Fraser Secondary Enrichment HiVeg Broth Base | 500 g | 61000-442 | Each |
| HiMedia Supplement Fraser Selective | 5 Packs | 71005-796 | Pack of 5 |
| VWR® FILTRA-BAG® with Safety Tab and Flat Wire Closure Seward Stomacher® Blender | 2.72 L | 10048-890 | Pack of 100 |
| VWR® E-Series Balance | 3,000 g | 10204-994 | Each |
| VWR® General Purpose Water Bath, Precalibrated | 20 L | 10796-926 | Each |
| VWR® Forced Air Microbiological Incubator | — | 89511-428 | Each |
| VWR® Storage/Media Bottles, Borosilicate | 500 mL | 89000-238 | Case of 10 |
| VWR® Square PETG Media Bottles | 1 L | 89132-056 | Pack of 22 |



Visit vwr.com/dupontbax for more information on BAX System instruments and PCR assays!