



**FOOD SAFETY LABORATORY
CORNELL UNIVERSITY**

Using BAX® to screen for *Listeria monocytogenes*

Created by: Reid Ivy, Sherry Roof and Yvonne Chan

Adopted from: USDA MLG 8A.01

**Detection and Isolation of *L. monocytogenes* from
Food and Environmental samples using the BAX®
system**

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**Authored by: Reid Ivy, Sherry Roof,
and Yvonne Chan**

Adopted from USDA MLG 8A.01

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APPROVED BY:

Dr. Martin Wiedmann

(date)

AUTHORED BY:

(Name)

(date)



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SECTION 1 INTRODUCTION

1.1 Purpose

The purpose of this document is to set forth **standard** guidelines for detection and isolation of *Listeria monocytogenes* from food or environmental samples using the BAX® system. This SOP is modified from USDA MLG 8A.01

1.2 Scope

This SOP applies to the Food Safety Lab, including the Laboratory for Food Microbiology and Pathogenesis of Foodborne Diseases.

SECTION 2 MATERIALS

Supplies

1. Food Sample or Environmental Sponge
2. Scale
- g. Stomacher
- h. Filter Stomacher bags
- i. Sterile sample preparation supplies (e.g. scalpels, knives, scissors, forceps, dishes, pans, or trays, pipet tips [filtered and non-filtered])
- j. BAX® assay for screening *Listeria monocytogenes* (Qualicon #17710609)
- k. MicroAmp optical 96-well reaction (Applied Biosystems N801-0560)
- l. Optical plate covers (Applied Biosystems 4311971)

Media

1. Modified University of Vermont broth (UVM)
2. Modified Oxford Agar



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3. MOPS-BLEB medium
 - a. Listeria Enrichment Broth
 - b. MOPS free acid (Sigma #1254)
 - c. MOPS sodium salt (Sigma #M9381)
4. LMPM



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SECTION 3 PROCEDURES

DAY 1:

3.1 Sample preparation

- a. Meat and poultry products: A 25 ± 1.0 g portion is used for raw and processed red meat, poultry and egg product testing. The test portion should be a composite representative of the entire sample.
 1. Intact retail packages must be disinfected at the incision sites immediately prior to incision for sampling. Appropriate disinfectants include but are not limited to ca. 3% hydrogen peroxide, ca. 70% ethanol or ca. 70% isopropanol. If the package does not appear to be clean, scrub gently using soapy water and rinse thoroughly prior to disinfection. A sterile scalpel may be helpful for cutting the packaging. Aseptically pull the packaging away to expose the product for sampling.
 2. Using a sterile scalpel, knife, spoon, chisel or other tool cut small pieces from representative sites on the sample to prepare a composite sample.
 3. Place 25 ± 1.0 g of the composite sample in a sterile Stomacher™ bag (i.e. filter bag recommended). If necessary, double-bag with a non-filter bag prior to stomaching (e.g. zip-lock bag).
 4. If analysis of the test portion is not to be initiated within 1 h, store at $\leq -10^{\circ}\text{C}$. Do not dilute the sample until ready to initiate analysis.

3.2 Primary Enrichment in UVM broth

- a. For all meat, poultry and egg product samples:
 1. To the 25 ± 1.0 g test portion, dispense 225 ± 5 ml (or 225 ± 5 g) of UVM broth. Stomach or blend for 2 ± 0.2 minutes. If blended, aseptically transfer the homogenate to an appropriate sterile container. Incubate the homogenate at $30 \pm 2^{\circ}\text{C}$ for 22 ± 2 h.



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- b. For environmental sponge samples:
 1. Aseptically transfer sponge to stomacher bag, add 225 ± 5 ml of UVM broth to each bagged sponge sample and stomach 2 ± 0.2 min.
 2. Incubate at $30 \pm 2^\circ\text{C}$ for 22 ± 2 h. Proceed to Section 3.3.
- a. Controls:
 1. Positive control:

From an overnight culture of 10403S (at least 18h at 37°C), complete 6 x 10-fold dilutions (Resulting suspension should be about 2×10^3 CFU/g). Inoculate 225 ml of UVM broth with 25 μl of the 10^{-6} tube. Resulting inoculum should be approx. 50 CFU.
 2. Negative control:

Include an un-inoculated 225 ml of UVM with the batch and complete all subsequent analyses.

DAY 2:

3.3 Secondary Enrichment and Direct Plating

- a. Transfer 0.1 ± 0.02 ml of the primary enrichment to 10 ± 0.5 ml of MOPS-BLEB. Incubate inoculated MOPS-BLEB tubes at $35 \pm 2^\circ\text{C}$ for 26 ± 2 h.
- b. Streak a MOX plate from the primary enrichment. Streak a loopful of UVM or a drop approximating 0.1 ml of the UVM over the surface of the plate. Alternatively, dip a sterile cotton-tipped applicator or equivalent into the UVM and swab 25-50% of the surface of a MOX plate. Use a 10 μl loop to streak for isolation from the swabbed area onto the remainder of the plate. Incubate the MOX at $35 \pm 2^\circ\text{C}$ for 26 ± 2 h.



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DAY 3:

3.4 Plating of secondary enrichment:

Streak a MOX plate from the secondary enrichment. Swab 25-50% of the surface of a MOX plate using a loopful of the MOPS-BLEB, a drop (approximating 0.100 ml) or a sterile cotton-tipped applicator. Use a 10 µl loop to streak for isolation from the swabbed area onto the remainder of the plate. Incubate the MOX at 35 ± 2°C for a minimum of 24 h.

3.5 Perform PCR-base detection of LM from secondary enrichment using BAX® System for Screening *L. monocytogenes*

An aliquot from the MOPS-BLEB secondary enrichment should be used for the BAX® system.

3.5.1 Lysate Preparation:

- a. **Preheat heating blocks (55° C and 95° C), chill PCR tubes**
- b. Prepare lysis reagent (use filter tips):
Add 150 µl of protease to one 12 ml bottle of lysis buffer. (To prepare less, the ratio is 12.5 µl of protease to 1 ml of lysis buffer. The buffer is stable for 2 weeks after the addition of protease. (Stored at 4° C)
- c. Label 1.5 ml centrifuge tubes. Aliquot 200 µl of lysis reagent to each tube.
- d. Aliquot 5 µl of the secondary enrichment to the corresponding tube.
- e. Heat tubes in heating block at 55° C for 60 minutes.
- f. Transfer tubes to a 95° C heat block, and heat for 10 minutes.
- g. Chill samples on ice for about 5 minutes. Samples must remain chilled until loaded on the instrument. Start the instrument so it is at a constant temperature.
- h. In the PCR prep room, aseptically transfer tablets to a 96-well optical plate. Put on optical plate cover.
- i. At your bench, hydrate the tablets from the BAX kit with 50 µl of lysate. Let stand 3-5 minutes.
- j. Spin down 96 well plate with table-top centrifuge, speed “6” until it reaches 3,200 rpm.

3.5.2 ABI 7000 Protocol for the BAX *L. monocytogenes* System:

- f. Turn on laptop and ABI Prism 7000 machine.
- g. Launch the 7000 SDS software from the desktop shortcut
- h. Select **File** → **New**.
- i. In the New Document dialog box, select **Absolute Quantitation** from the **Assay** menu and select **Browse...** for the “BAX Lmono Template” from Sherry’s Folder. Leave the rest of the choices as they appear.
- j. Click **OK**.

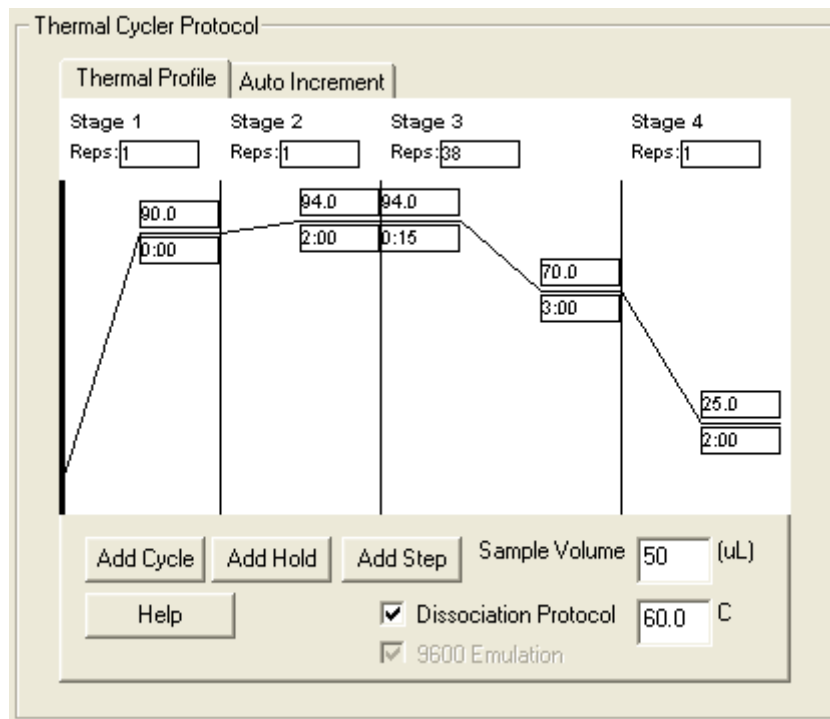


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- f. To make changes to the Plate Document, select **View** → **Well Inspector**. (Note: make sure the **Detector** is **BAX**, the **Reporter** is **SYBR**, the **Quencher** is **None**, and the **Passive Reference** is **None** for all wells containing sample.)
 1. For the **unknown sample(s)** and/or **positive control**, type a name for the sample in the **Sample Name** field, if necessary. Make sure you designate **two** wells for each sample.
 2. For the **negative control**, make sure you have **two** wells.
 3. To delete information in a well, check the **Omit Well** box or uncheck detector and delete the sample name.
- g. Close the Well Inspector.
- h. Select **Instrument** tab. Make sure the **Thermal Cycling Protocol** has the following settings:



- i. Save the plate document.
- j. Place plate (**with foam pad on top**) into the machine.
- k. In the **Instrument** tab, click on **Start**.
- l. After thermocycling is complete, save results.
- m. To analyze the run:
 1. Select the **Dissociation** tab. Select **Data Type: Derivative**. Click on a well to be analyzed.
 2. A sample **positive** for *L. monocytogenes* should have **two peaks**. One peak at ~ **78°C** for the internal positive control (IPC) and another at ~

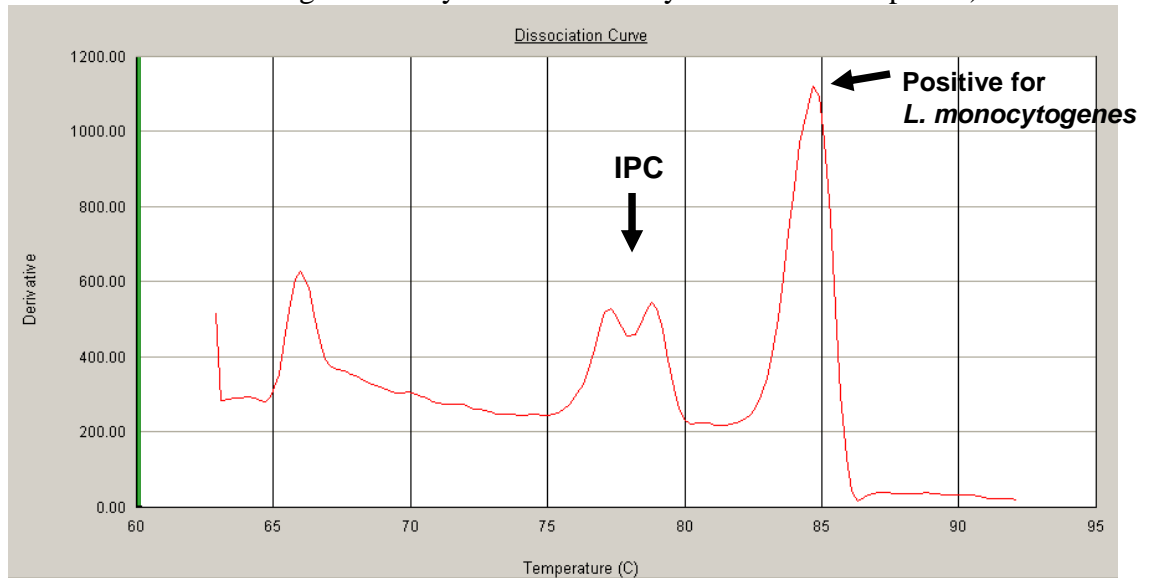


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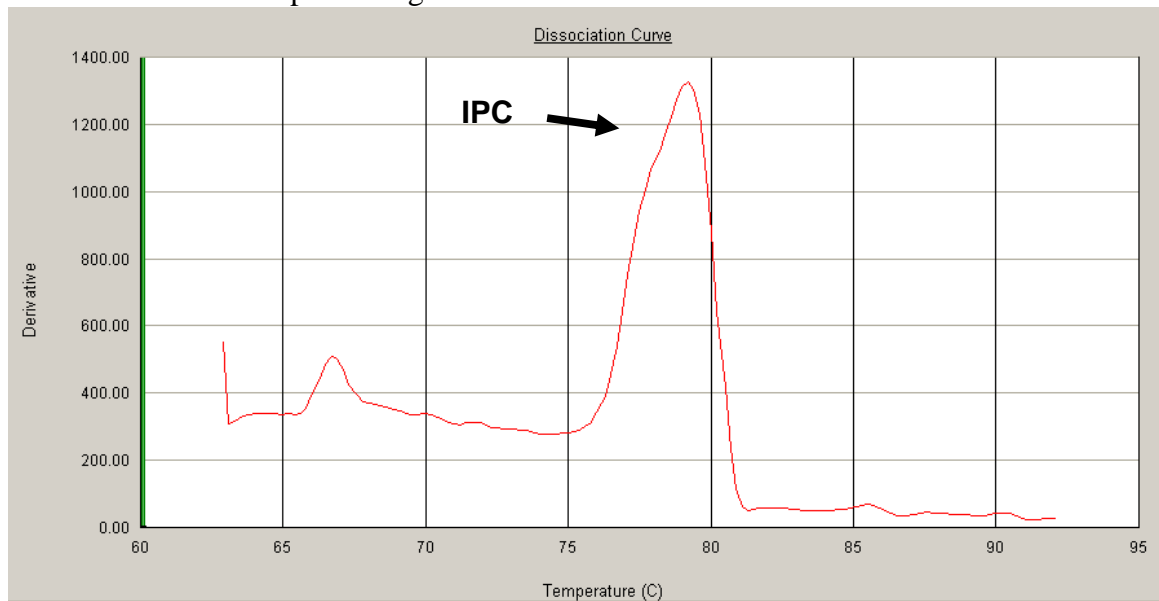
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84°C for the *L. monocytogenes* PCR product. (Note: Peak height can vary and the IPC may have two small peaks.)



3. A sample **negative** for *L. monocytogenes* should have **one peak** at ~**78°C** representing the IPC.



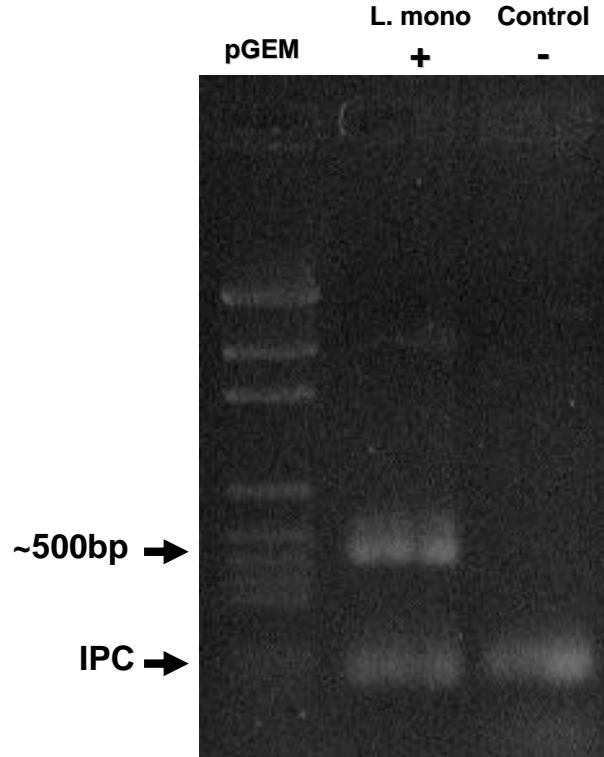
4. If the results are unclear, run a 1.5% agarose gel electrophoresis with 10µl of PCR product, 10µl negative control, and 5µl of pGEM DNA marker.



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SECTION 4 Interpretation

4.0 Interpretation of BAX results and results of culture-based detection from Primary Enrichment

Initial results will be reported based on results of the BAX assay and the Oxford plating from the primary enrichment as detailed in the table below.

INTERPRETATION OF RESULTS	
RESULT	ACTION
(A) BAX neg/ no Listeria like colonies on Oxford plating from primary enrichment	Report sample negative
(B) BAX neg/Listeria like colonies on Oxford plating from primary enrichment*	(1) Sub-culture 4 Listeria like colonies on to LMPM (2) if ≥ 1 LM like colony is detected, report sample as LM-positive and ribotype and freeze LM isolates (3) if no colonies show LM-like profiles report sample as LM-negative
(C) BAX pos/Listeria-like colonies on Oxford plating from primary enrichment*	(1) Sub-culture 4 Listeria like colonies to LMPM (2) if ≥ 1 LM like colony is detected, report sample as LM-positive ; ribotype and freeze LM isolates. (3) if no colonies show LM-like profiles, report sample as LM-negative
(D) BAX pos/no Listeria like colonies on Oxford plating from primary enrichment	(1) If no Listeria like colonies on plating from secondary enrichment, report sample as negative (2) If Listeria-like colonies on plating from secondary enrichment, sub-culture 4 Listeria like colonies from secondary enrichment on to LMPM (3) If ≥ 1 LM like colony is detected, report sample as LM-positive ; ribotype and freeze LM isolates. (4) if no colonies show LM-like profiles, report sample as LM-negative
(E) BAX failure (i.e., no positive band & positive control failed)/no Listeria like colonies on Oxford plating from primary or secondary enrichment	Report sample negative
(F) BAX failure (i.e., no positive band & positive control failed)/Listeria like colonies on Oxford plating from primary or secondary enrichment	(1) Sub-culture 4 Listeria like colonies from plating of primary enrichment onto LMPM (sub from platings of secondary enrichment if primary enrichment has < 5 Listeria like colonies) (2) If ≥ 1 LM like colony is detected, report sample as LM-positive ; ribotype and freeze LM isolates. (3) if no colonies show LM-like profiles, report sample as LM-negative

*Sub-streak up to 4 colonies for each positive sample on LMPM.
Incubate 48 hours at 37°C



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4.1. Confirmation and further characterization of *L. monocytogenes* like colonies on LMPM:

- a. For every colony sub-streaked that grows blue-green, freeze one colony.
- b. Follow FSL protocol for assigning numbers and freezing isolates (see protocol on internal web page.)
- c. One isolate per sample should be ribotyped. When entering the information into Pathogen Tracker (see protocol on internal web page), note that the isolate is "representative".
- d. In analytical runs where the positive control tests negative, the reserve samples will be retested. If the retested sample is negative, the cultural method is completed by streaking the secondary enrichment MOPS-BLEB medium onto MOX plates. Patch 4 colonies from the MOX plates (either from primary or secondary enrichment; use colonies from plating of primary enrichment if available) onto LMPM (see results E and F in table above).



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SECTION 5 TROUBLESHOOTING

New protocol. Troubleshooting information will be included as needed.

SECTION 6 REFERENCES

1. Isolation and Identification of *Listeria monocytogenes* from Red Meat, Poultry, Egg, and Environmental Samples. USDA. MLG 8.04. Available at:
http://www.fsis.usda.gov/Ophs/Microlab/Mlg_8_04.pdf
2. FSIS Procedure for the Use of *Listeria monocytogenes* BAX® Screening Test. USDA. MLG 8A.01. Available at: http://www.fsis.usda.gov/ophs/Microlab/Mlg_8A_01.pdf