Laboratory Procedure **MFLP-28**
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HEALTH PRODUCTS AND FOOD BRANCH

OTTAWA

THE QUALICON BAX® SYSTEM METHOD FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* IN A VARIETY OF FOOD

1. APPLICATION

This method is applicable to the detection of *Listeria monocytogenes* in a wide variety of food, including raw meats, processed meats, seafood, fresh produce/vegetables, cultured and non-cultured dairy, egg and egg products, and fruit juices.

2. DESCRIPTION

The BAX® system is a convenient yes/no screening tool that uses Polymerase Chain Reaction (PCR) technology for rapid amplification and fluorescent detection. Food processors and associated laboratories can use the BAX® system as a quick method for accurately detecting *Listeria monocytogenes* in a wide variety of foods. Following 22-26 hours primary enrichment and 18-24 hours secondary enrichment, sample preparation involves about 1 hour of user time, and the automated procedure delivers reliable results about 4 hours later. BAX® system validation studies used the USDA-FSIS enrichment method for meat, poultry and eggs; the AOAC method 993.12 for dairy; and the FDA-BAM method for other food types (see Section 8: Reference Methods). The BAX® system is designed for use by qualified lab personnel who follow standard microbiology practices.

3. PRINCIPLE

The BAX® System uses the Polymerase Chain Reaction (PCR) to amplify a specific fragment of bacterial DNA, which is stable and unaffected by growth environment. The fragment is a genetic sequence that is unique to *Listeria monocytogenes*, thus providing a highly reliable indicator that the organism is present. The automated BAX® system then uses fluorescent detection to analyze PCR product for positive or negative results.

PCR is a powerful means for quickly providing millions of copies of a specific DNA fragment. In a typical application, sample DNA is combined with polymerase, nucleotides and primers that are specific for a given nucleotide sequence. This mixture then undergoes a series of timed heating and cooling cycles. Heating denatures or separates the DNA into single strands, then as the mixture cools, primers recognize and anneal to the target DNA sequence. DNA polymerase then uses nucleotides to extend the primers, thereby creating two copies of the target DNA fragment. Repeated cycles of denaturing, annealing, and extending produce exponential increases in the number of target DNA fragments within a matter of hours. If the target sequence is not present, no detectable amplification takes place.

The BAX® system simplifies this process by combining the primers, polymerase, nucleotides and positive control into a single sample tablet that is already packaged inside the PCR tubes. Additionally, the automated fluorescent detection allows for closed-tube testing, eliminating the potential for carry-over contamination with amplified DNA.

Published on the Food Directorate's (Health Canada's) website at http://www.hc-sc.gc.ca/food-aliment.

4. DEFINITION OF TERMS

See Appendix A of Volume 3.

5. COLLECTION OF SAMPLES

See Appendix B of Volume 3.

6. MATERIALS AND SPECIAL EQUIPMENT

1) Supplied with kit – (no.17710609; sufficient for 96 tests – DuPont Qualicon, phone: 1-800-863-6842, fax: 302-695-5301)

2 bags	PCR sample tablets, packaged 1 tablet per PCR tube in 12 strips of 8 tubes. The tablets include the reagents needed for the test reaction as well as an internal positive control (which eliminates the need to run a separate QC reaction). Tablets are 7.6 ± 0.1 mg.
1 bag	Optical caps, 12 strips of 8 caps.
2 bottles	Lysis buffer, pH 8.35 ± 0.05 @ 25 °C. 12 ml/bottle. Used to prepare working lysis reagent.
1 vial	Protease solution, 400 µL/vial. Used to prepare working lysis reagent

2) Enrichment broths

Primary enrichment - varies by food type (see Section 7)
UVM broth
Demi-Fraser broth
Selective enrichment broth
Enrichment broth
Universal preenrichment broth

Secondary enrichment – MOPS-BLEB

3) Materials (included with BAX® system start-up package)

Equipment

Stomacher
Incubator
Other (included with BAX® system start-up package)
Cycler/detector with verification plates
Computer workstation with Microsoft Windows ® operating system, BAX® system application, and printer
Dry block heaters with thermometers inserts for lysis tubes
Capping/decapping tools
Various pipettes for reagent and sample transfers
Cooling block with inserts for lysis tubes and PCR tubes
PCR tube holders

Supplies
Lysis tubes with caps and rack
Tips for pipettes
Powder-free nitrile gloves

4) BAX® system *User Guide*

7. PROCEDURE

7.1 Collect and enrich samples

Prepare samples according to a standard method for the food type, as follows:

Sample type	Primary enrichment	Secondary enrichment
Raw meat and poultry	Blend 25 g sample with 225 mL Demi Fraser broth. Incubate at 30°C for 22-26 hours.	Add 100 µL enriched sample to 9.9 ml pre-warmed MOPS-BLEB broth. Incubate at 35°C for 18-24 hours.
Other meat and poultry	Blend 25 g sample with 225 mL UVM broth. Incubate at 30°C for 20-24 hours	Add 100 µL enriched sample to 9.9 ml pre-warmed MOPS-BLEB broth. Incubate at 35°C for 18-24 hours.
Smoked fish	Blend 25 g sample with 225 mL Universal Preenrichment broth. Incubate at 35°C for 22-26 hours.	Add 1 ml enriched sample to 9 ml pre-warmed MOPS-BLEB broth. Incubate at 35°C for 18-24 hours.
Dairy products	Blend 25 g sample with 225 mL Complete Selective Enrichment broth. Incubate at 30°C for 48 hours	Add 100 µL enriched sample to 9.9 ml pre-warmed MOPS-BLEB broth. Incubate at 35°C for 18-24 hours.
Other foods	Blend 25 g sample with 225 mL Enrichment broth. Incubate at 30°C for 4 hours before adding selective agents acriflavin, nalidixic acid and cycloheximide. Continue incubating at 30°C for another 44 hours	Add 100 µL enriched sample to 9.9 ml pre-warmed MOPS-BLEB broth. Incubate at 35°C for 18-24 hours.

7.2 Prepare equipment (Refer to BAX® System *User Guide* for details)

7.2.1 Make sure cooling blocks have been refrigerated overnight.

7.2.2 Warm up heating blocks. Check that temperatures are set to 55ºC and 95ºC.

7.2.3 Initialize BAX® system cycler/detector (perform verification, if prompted).

7.2.4 Create a rack file.

7.2.5 Select RUN FULL PROCESS in the menu bar to warm up the cycler/detector.

7.3 Prepare samples

7.3.1 Lyse samples

7.3.1.1 Arrange required number of lysis tubes (one for each sample and one for the “blank”) in the rack according to the rack file.

7.3.1.2 Prepare lysis reagent by pipetting 150 µL of protease into one 12-mL bottle of lysis buffer.

7.3.1.3 Add 200 µL of lysis reagent to each lysis tube.

7.3.1.4 Transfer 5 µL of enriched sample to the corresponding lysis tube.

7.3.1.5 Secure the caps and heat the tubes at 55ºC for 60 minutes, then at 95ºC for 10 minutes.

7.3.1.6 Place the lysis tubes in cooling block for at least 5 minutes.

7.3.2 Prepare samples for PCR

7.3.2.1 Place PCR tube holder onto PCR cooling block.

7.3.2.2 Arrange one PCR tube per sample in the holder.

7.3.2.3 Remove and discard lid from one strip of tubes at a time.

7.3.2.4 Using multi-channel pipette, transfer 50 µL of each lysed sample into a corresponding PCR tube.

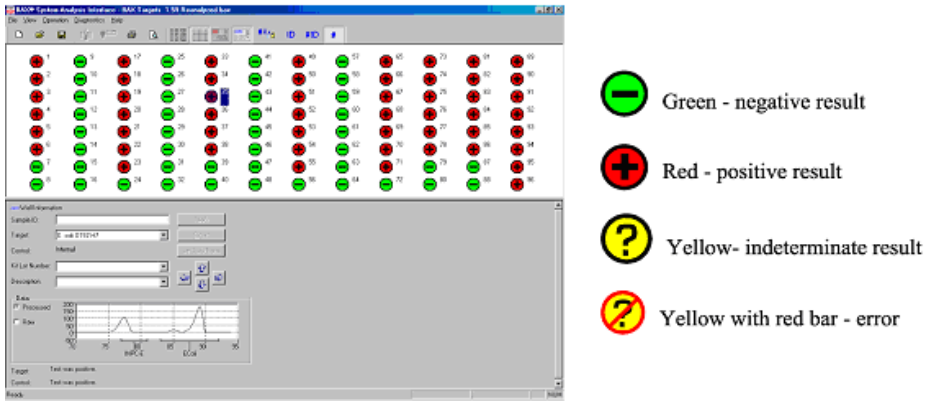
7.3.2.5 Cover tubes with a new optical cap strip and secure tightly. Repeat for all samples.

7.3.2.6 Take the entire cooling block to the cycler/detector. Samples should remain in the cooling block until the cycler/detector is ready for loading, but no more than 30 minutes after tablet hydration.

7.4 Process samples

Follow the screen prompts of the PCR Wizard to load your samples, run the program and unload your samples, as specified in the *User Guide*.

7.5 Review results



7.6 Confirm positive results

Positive samples must be confirmed culturally as describe in MFHPB-30 (see Section 8: Reference Methods). Spread plate to specified selective agars and confirm biochemically and serologically.

8. Reference Methods

8.1 Health Canada, Health Products and Food Branch, Food Directorate, Bureau of Microbial Hazards. Compendium of Analytical Methods, Volume 2 [Internet] Ottawa: The Branch; c2001 [suppl 2002 March]. MFHPB-30. Isolation of *Listeria* monocytogenes from all Food and Environmental Samples [about 15 screens]. Available from http://www.hc-sc.gc.ca/food-aliment/mh-dm/mhe-dme/compendium/volume_2/e_index.html

8.2 Official Methods of Analysis (2002), 17th edition. AOAC INTERNATIONAL, Gaithersburg, MD, section 993.12.

8.3 US Department of Agriculture, Food Safety and Inspection Service, Office of Public Health and Science. Microbiology Laboratory Guidebook [Internet]. Washington: The Dept; c2002 [rev 2002 Apr 29; cited 2002 July 22]. Chapter 8, Isolation and Identification of *Listeria monocytogenes* from

Red Meat, Poultry, Egg and Environmental Samples [about 20 screens]. Available from http://www.fsis.usda.gov/OPHS/microlab/mlgchp803.pdf

8.4 US Food & Drug Administration, Center for Food Safety & Applied Nutrition. Bacteriological Analytical Manual Online [Internet]. Washington: The Admin; c2001 [rev 2001 April; cited 2002 July 22]. Chapter 10, *Listeria monocytogenes* [about 12 screens]. Available from http://www.cfsan.fda.gov/~ebam/bam-10.html