Detection and Isolation of Listeria spp, L. monocytogenes, and Salmonella from FPDL Dairy Environmental Samples

FILE NAME: FPDL Sample Processing Protocol.doc

Authored by: Maureen Gunderson
Updated by: Sarah Beno
Adopted from: FDA BAM
Last Modified on: 01/29/2013
Approved by: Martin Wiedmann

EFFECTIVE DATE: Date of Approval

APPROVED BY:

_____ _____
Dr Martin Wiedmann (date)

AUTHORED BY:

_____ _____
(Name) (date)

SECTION 1: INTRODUCTION

1.1. Purpose

The purpose of this document is to set forth standard guidelines for detection and isolation of *Listeria spp*, *L. monocytogenes,* and *Salmonella* from environmental samples.

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

2.1 Supplies

A. Laboratory Stomacher

B. Components for *sigB Listeria monocytogenes* PCR assay

C. Components for *invA* Salmonella PCR assay

D. Overnight culture of *L. mono* 10403S (positive control)

E. Overnight culture of *Salmonella* FSL S5-370 (positive control)

2.2 Media

A. *Listeria monocytogenes and Listeria spp.*

1. Buffered Listeria Enrichment Broth Base (BLEBB)

2. Oxford Agar Base (BD 222510)

3. *Listeria* Selective Enrichment Supplement (LSES). Use Oxoid SR0149A or prepare supplement using Cyclohexamide, Nalidixic Acid, Acroflavin, and Sodium Hydroxide.

4. Modified Oxford Supplement (Oxoid SR0206E)

5. *Listeria monocytogenes* plating medium (LMPM), R & F Laboratories 0550M

B. *Salmonella*

1. Lactose Broth
2. Rappaport Vassiliadis (RV) Broth medium
3. Tetrathionate (TT) Broth medium
4. Iodine-Potassium Iodide Solution
5. Xylose Lysine Desoxycholate (XLD) Agar
6. CHROMagar™ Salmonella medium

SECTION 3: MEDIA PREPARATION

3.1 Media for Listeria Isolation

A. Buffered Listeria Enrichment Broth Base (BLEBB)

1. Suspend 48 g powder in 1 L of purified water
2. Autoclave at 121° C for 15 minutes

B. Modified Oxford Agar (MOX)

1. Suspend 27.75 g of Oxford Agar Base in 500 ml of purified water. Mix well. Autoclave at 121° C for 15 minutes
2. Cool to 55° C and aseptically add the contents of one vial of Modified Listeria Selective Supplement (Oxoid SR0206E).
3. Mix well and pour into Sterile Petri Plates. Can be made on Master-Clave Auto Plate Pourer. MOX plates are light sensitive.

C. Listeria Selective Enrichment Supplement (50 ml).

1. The ingredients for this supplement should be handled with extreme caution. Wear safety goggles, mask, lab coat and gloves. MSDS is available for each ingredient. Weigh all of the ingredients on the analytical balance. Remaining work should be done in the fume hood.
2. Nalidixic Acid must be dissolved in a basic solution. Using low heat, dissolve 180 mg of Sodium Hydroxide (NaOH) in 15 ml sterile DH_2O. Add 450 mg of Nalidixic Acid. Carefully pipette the mixture up and down to dissolve.

3. Add 112.5 mg of Acriflavin HCL to 5 ml sterile DH_2O. Carefully pipette the mixture up and down to dissolve.

4. Dissolve 562.5 mg of Cycloheximide in 5 ml of methanol. Carefully pipette the mixture up and down to dissolve.

5. Combine the above mixtures in a glass graduated cylinder. Bring the volume to 50 ml using sterile DH_2O. Protect completed mixture from light.

D. *Listeria monocytogenes* Plating Medium (LMPM)

1. Suspend 30.3 g of powder in 485 ml of purified water. Mix well. Autoclave at 121° C for 15 minutes.

2. Cool to 55° C and add LMPM supplements per manufacturer's instructions. Mix well and pour into Sterile Petri Plates. Can be made on Master-Clave Auto Plate Pourer. LMPM Plates are light sensitive.

3.2 Media for Salmonella Isolation

A. Lactose Broth (Difco)

1. Suspend 13 g powder in 1 Liter of purified water.

2. Autoclave at 121° C for 15 minutes.

B. Rappaport Vasiliadis Broth

1. Suspend 26.6 g powder in 1 Liter of purified water.

2. Aliquot 9.9 ml RV broth to 16 x 150 glass tubes (one for each sample plus controls) and autoclave at 121° for 15 minutes. Tubes should be used within 1 month of preparation.

C. Tetrathionate (TT) Broth

1. TT Broth Base: Suspend 77 g TT Broth Base (Oxoid CM0029) in 1 L purified water. Bring to a boil. Do NOT autoclave. Pipet aseptically into 18 x 150 glass culture tubes. Broth can be stored at 2° to 8° for several weeks without the iodine solution.

2. Prepare Iodine Solution: Add 6 g Iodine and 5 g Potassium Iodide to 20 ml purified water.

3. Just prior to using the TT Broth Tubes, add 180 ul Iodine Solution to each TT Tube. Once Iodine solution is added, tubes must be used within a few hours.

D. XLD Agar Plates

1. Add 57 g Difco™ XLD Agar to 1 Liter of purified water.

2. Heat just to boiling. DO NOT AUTOCLAVE. Pour into sterile petri plates. XLD Plates are light sensitive.

E. CHROMagar™ Salmonella

1. Suspend 34.9 g in 1 L of purified water. Bring just to a boil. Do not heat to more than 100° C.

2. Cool to 45 to 50° C. Pour into sterile petri plates. CHROMagar™ Salmonella plates are light sensitive.

SECTION 4: SAMPLE PREPARATION AND PROCESSING

DAY 0

4.1 Sample preparation and handling.

A. Samples should be stored at all times either at 4° C or on ice.

B. For quarterly sampling, two (2) sample sponges for each quarterly sample site will be collected or shipped from the plant. One set will be used to detect *Listeria monocytogenes* and *Listeria spp.* and the other will be used to detect *Salmonella*. For regular monthly sampling sites (*Listeria* only), one sample sponge for each monthly site will be collected or shipped from the plant.

C. A list showing sample site number and description will be provided. The numbered sample bags must be verified with this list.

4.2 Primary Enrichment for isolation of Listeria.

A. Add 90 ml BLEBB (WITHOUT LSES Supplement) to each Listeria sponge sample bag. Stomach for 1 minute
1. 25 ml of raw milk is combined with 225 ml of BLEBB in a whirlpack bag.

B. Prepare Listeria positive and negative controls.

1. Positive Control: Add 90 ml BLEBB to sterile sample bag with sponge. Select one colony of *Listeria monocytogenes* 10403S from pre-prepared BHI plate and inoculate the BLEBB.

2. Negative Control: Add 90 ml BLEBB to sterile sample bag with sponge.

C. Incubate enriched samples (without LSES Supplement) for 4 hours at 30° C.

D. Prepare Listeria Selective Enrichment Supplement (LSES).

E. After samples have finished the initial 4 hour incubation, remove from the incubator and add 360 µl of LSES to each sample. Return to the incubator for an additional 20 hours.
1. Add 900 µl of LSES to each raw milk sample.

4.3 Primary Enrichment for isolation of Salmonella

A. Add 225 ml Lactose Broth to each Salmonella sponge sample bag. Stomach for 1 minute.

B. Prepare Salmonella positive and negative controls.

1. Positive Control: Add 225 ml Lactose Broth to sterile sample bag with sponge. Select one colony of Salmonella, serotype Typhimirium FSL S5-370 from pre-prepared BHI plate, and inoculate the Lactose Broth

2. Negative Control: Add 225 ml Lactose Broth to sterile sample bag with sponge

C. Incubate at room temperature for 4 hours

D. Move to 35° C incubator. Incubate for 24 hours.

DAY 1

4.4 Streaking for isolation of Listeria (24 hour enrichment).

A. After samples have incubated for 24 hours, streak them for isolation using 50 µl aliquots of each sample. Streak each sample on one MOX plate and one LMPM plate.

B. Incubate MOX plates at 30° C for 48 hours. Incubate LMPM plates at 35° C for 48 hours.

C. Return samples to the incubator for another 24 hour incubation period. (Total sample incubation time is 48 hours)

4.5. Secondary enrichment for isolation of Salmonella

A. Add Iodine-Potassium Iodide Solution to TT broth tubes.

B. Add 1 ml of each sample enrichment to TT Broth tubes

C. Add 100 µl of each sample enrichment and controls to RV Broth tubes.

D. Place tubes in 42° C water bath (with shaker) for 24 hours

DAY 2

4.6. Streaking for isolation of Listeria (48 hour enrichment).

A. After samples have incubated for a total of 48 hours, streak them for isolation using 50 µl aliquots of each sample. Streak each sample on one Modified Oxford (MOX) plate and one LMPM plate.

B. Incubate MOX plates at 30° C for 48 hours. Incubate LMPM plates at 35° C for 48 hours.

4.7. Streaking for isolation of Salmonella

A. After samples have incubated in RV and TT broth tubes for 24 hours, streak for isolation using 50 µl aliquots of each tube. Streak each RV sample on one XLD plate and one CHROMagar ™ plate. Streak each TT Broth sample on XLD and CHROMagar™ .

B. Incubate plates at 35° C for 24 hours.

DAY 3

4.8 Processing Positive results - Listeria (24 hour enrichment)

A. Record results from 24 hour enrichments plated on MOX and LMPM. Results will be recorded on sample list sheet. See appendix A and B for examples of monthly and quarterly sheets).

B. Sub-streak positive results. (Since preference for colony selection is given to the 24 hour plate, positive results may be streaked without reading the 48 hour plate).

1. Sub-streak up to four (4) colonies from LMPM or MOX plates that demonstrate typical *Listeria monocytogenes* morphology to LMPM plates.

2. Sub-streak up to two (2) white colonies from LMPM or colonies from MOX plates that demonstrate typical *Listeria spp*. morphology to LMPM plates

C. Notify Dairy Operations personnel of putative positives via email. A current listing of contacts is attached as Appendix G.

4.9 Processing Positive Results - Salmonella

A. Record results from XLD and CHROMagar™ plates. Worksheet example for recording quarterly results is attached as Appendix C.

B. Substreak a total of four (4) putative salmonella colonies. If colonies appear on both XLD and CHROMagar™ plates, select two (2) colonies from each plate. If colonies appear on just one type of media, select up to four (4) from that plate.

C. Notify Dairy Operations personnel of putative positives via email. (See appendix G)

DAY 4

4.10 Processing Positive Results - Listeria (48 hour enrichment)

A. Record results from 24 hour enrichments plated on MOX and LMPM. Results will be recorded on sample list sent from Dairy. See appendix A and B for examples of monthly and quarterly sheets).

B. Sub-streak positive results that were not identified from 24 hour samples.

C. Sub-streak four (4) colonies from each sample that demonstrated typical *Listeria monocytogenes* morphology. Sub-streak to LMPM plates

D. Sub-streak two (2) white colonies from LMPM plates or colonies from MOX plates that demonstrate typical *Listeria spp*. morphology to LMPM.

E. View plates that were sub-streaked from 24 hour enrichment plates.

F. If the 48 hour plating reveals a positive that was not found in the 24 hour plating, immediately notify Dairy operations personnel of putative positive.

DAY 5+

4.11 Confirmation and Storage of Positive Results

A. Record results from LMPM plates (48 hour enrichment) that were sub-streaked on day 4.

B. Perform modified *sigB* PCR to confirm *Listeria monocytogenes* and *Listeria spp.* colonies. Once confirmed, assign FSL isolate numbers and prepare for cryogenic storage in accordance with protocol. Select 4 colonies from positive site plates. Preference should be given to the 24 hour plates (LMPM or MOX).

C. Perform invA PCR to confirm Salmonella colonies. Once confirmed, assign FSL isolate numbers and prepare for cryogenic storage. Select 4 colonies for storage.

4.12 Report final results to FPDL/Dairy

A. Record final sampling results. Worksheet for recording final monthly results is attached as Appendix D. Worksheet for recording final quarterly results is attached as Appendix E.

B. Print worksheet to compare against written copies.

C. Give to another person to double check with a colored pen.

D. Attach worksheet to email and forward to appropriate personnel.