	FOOD SAFETY LAB / MILK QUALITY IMPROVEMENT PROGRAM *Standard Operating Procedure*	

Title: Detection and Isolation of Listeria and Salmonella from environmental samples			
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Detection and Isolation of Listeria and Salmonella from environmental samples

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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* spp. and *L. monocytogenes*, or *Salmonella* from environmental samples, and the preparation of the media necessary for this task. This is a modified version of the FDA BAM methodology.
The SOP include specific "Notes" for testing samples from the Cornell dairy plant; these are indicated in red

1.2 Scope

This SOP applies to members of the Food Safety Laboratory, the Milk Quality Improvement Program Laboratory, the Worobo Laboratory, the Alcaine Laboratory, the Snyder Laboratory, as well as visiting lab members.

1.3 Safety

L. monocytogenes and *Salmonella* are BSL-2 human pathogens. Take the appropriate precautions necessary for handling materials exposed to BSL-2 pathogens. Enrichments for BSL-2 pathogens are to be handled as if they contain BSL-2 organisms.

Cyclohexamide, Nalidixic Acid, Acroflavin, and Sodium Hydroxide, components of the LSES supplement, can be a hazardous to health and should be handled with extreme caution in a fume hood to avoid exposure.

Iodine Potassium-Iodide, a component of the TT media broth, can be hazardous to health. Preparation of the stock solution should be performed in a fume hood. Appropriate precautions should be taken while handling these substances.

SECTION 2 MATERIALS

Supplies

- Laboratory Stomacher
- BHI plate with isolated colonies of Δ*actA L. monocytogenes* isolate (positive control) – FSL R3-0001.
- BHI plate with isolated colonies of *Salmonella* serotype Typhimurium isolate (positive control) – FSL S5-0370.
- Sterile sample bags with sponge
- dH_2O
- 10ul yellow loops

Media

*Instructions for preparing media and media components can be found in Appendix A.

- For *Listeria monocytogenes* and *Listeria* spp. enrichment:
  - Buffered Listeria Enrichment Broth (BLEB), made from Buffered Listeria Enrichment Broth Base according to manufacturer's instructions
  - Modified Oxford Agar
    - Oxford Agar Base - BD 222510
    - Modified Oxford Supplement - Oxoid SR0206E
  - Listeria Selective Enrichment Supplement (LSES) - Oxoid SR0149A
  - *Listeria monocytogenes* plating medium (LMPM) - R & F Laboratories 0550M
- For *Salmonella* enrichment:
  - Lactose Broth
  - Rappaport Vassiliadis (RV) Broth medium (9.9ml per 16x150 glass tube)
  - Tetrathionate (TT) Broth medium (9ml per 18x150 glass tube)
  - Iodine-Potassium Iodide Solution
  - Xylose Lysine Desoxycholate (XLD) Agar
  - CHROMagar™ Salmonella medium

SECTION 3 PROCEDURES

**Sections labeled "SALMONELLA" can be skipped when only doing monthly Listeria testing.*

DAY 0

3.1 Sample preparation and handling.

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

Notes for Cornell Dairy Plan sampling: 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 as determined by sampler will be collected or shipped from the plant. An electronic and paper copy of the Sample List showing sample site number and description will be provided. The numbered sample bags must be verified with this list.

3.2 LISTERIA - Primary Enrichment for isolation of Listeria.

A. Add 90 ml of room temperature BLEB (WITHOUT LSES Supplement) to each *Listeria* sponge sample bag.

1. If the environmental sample is raw milk, combine 25 ml of raw milk with 225 ml of BLEB in a whirlpack bag.

B. Prepare Listeria positive and negative controls.
1. Negative Control: Add 90 ml BLEB to a sterile sample bag with sponge.
2. Positive Control: Add 90 ml BLEB to a sterile sample bag with sponge. Select one colony (grab approx. 1/3-1/2 of colony) of ΔactA *L. monocytogenes* isolate – FSL R3-0001 from the pre- prepared BHI plate and inoculate the BLEB in the

bag. *Note: ideally the positive control would be inoculated with a reproducible small amount of L. monocytogenes, such as an overnight culture diluted to allow for inoculation of the positive control with about 100 CFU of Listeria monocytogenes*

(a) Store the positive control bag next to the negative control to assure the procedural problems with cross contamination will be detected.

C. Stomach the enrichment bags one at a time for 1 minute at 260 RPM.

The bags should not be rolled down or sealed before stomaching or they will rupture. The wire tabs at the top of the bags should hang outside the machine when the bag is inserted.

D. Incubate the enrichment bags (WITHOUT LSES Supplement) for 4 hours at 30°C in a secondary container (e.g., large plastic tupperware).

E. Prepare Listeria Selective Enrichment Supplement (LSES) by adding 10ml of dH_2O to the powdered supplement bottle (SR0149A) and vortexing until thoroughly mixed. It is normal for undissolved dark particulate to remain.

1. If the premade SR0149A preparation is unavailable, instructions for preparing the supplement are detailed in Appendix A.

F. Taking care to avoid touching the opening of the bags, roll the wire tabs down and fold in to seal the bags.

G. After samples have finished the initial 4 hour incubation, remove from the incubator and add 360 µl of LSES to each bag. Return bags to the incubator for an additional 20 hours. Bags should be opened and closed using the tabs without touching the opening of the bag.

Note: For raw milk sample or other samples that represent a final volume of about 250 ml, add 900 µl of LSES.

3.3 SALMONELLA - Primary Enrichment for isolation of Salmonella

A. Add 90 ml of room temperature Lactose Broth to each *Salmonella* sponge sample bag.

B. Prepare *Salmonella* positive and negative controls.

1. Positive Control: Add 90 ml Lactose Broth to a sterile sample bag with sponge. Select one colony (picking up 1/3-1/2 of colony) of Salmonella serotype Typhimurium – FSL S5-0370 from the pre-prepared BHI plate and inoculate the Lactose Broth in the bag.

2. Negative Control: Add 90 ml Lactose Broth to a sterile sample bag with sponge.
   (a) Store the positive control bag next to the negative control to assure the procedural problems with cross contamination will be detected.

C. Stomach the enrichment bags one at a time for 1 minute at 260 RPM.

1. The bags should <u>not</u> be rolled down or sealed until after stomaching or they will rupture. The wire tabs at the top of the bags should hang outside the machine when the bag is inserted.

D. Taking care to avoid touching the opening of the bags, roll the wire tabs down and fold in to seal the bags.

E. Incubate the enrichment bags at <u>room temperature</u> for 4 hours in a secondary container (e.g., large plastic tupperware).

F. Move the bags in the container to a 35°C incubator. Incubate for 24 hours.

DAY 1

3.4 LISTERIA - Streaking for isolation of Listeria (24 hour enrichment)

A. After *Listeria* samples have incubated for 24 hours, aseptically open each bag and transfer 50 µl aliquots of each sample enrichment to one MOX plate and one LMPM plate per sample. Streak each plate for isolation with yellow loops. *Make sure you also perform this procedure for positive and negative control samples; perform this procedure with positive control sample first followed by the negative control sample (this will assure that any procedural issues that lead to cross contamination are detected).*

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

C. Aseptically close the enrichment bags and return them to the incubator for another 24 hour incubation period. (Total sample incubation time is 48 hours)

3.5 SALMONELLA - Secondary enrichment for isolation of Salmonella

A. Preheat a shaking waterbath to 42°C.

B. Add 180 ul Iodine-Potassium Iodide Solution to each TT broth tube. After adding, these tubes should be used within a few hours.

   1. If the stock solution of Iodine-Potassium-Iodide needs to be prepared, see Appendix A.

C. Add 1 ml of each *Salmonella* sample enrichment and control to TT Broth tubes.

D. Add 100 µl of each *Salmonella* sample enrichment and controls to RV Broth tubes.

A. Note: *perform C and D above with positive control sample first followed by the negative control sample (this will assure that any procedural issues that lead to cross contamination are detected).*

B. Place the inoculated TT and RV tubes in a 42°C water bath (with shaker on) for 24 hours.

C. Store the enrichment bags at 4°C until final results are confirmed.

DAY 2

3.2 LISTERIA - Streaking for isolation of Listeria (48 hour enrichment)

A. After *Listeria* samples have incubated for 48 hours, transfer 50 µl aliquots of each sample enrichment to one MOX plate and one LMPM plate per sample. Streak each plate for isolation with yellow loops.

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

C. Store the enrichment bags at 4°C until final results are confirmed.

3.3 SALMONELLA - Streaking for isolation of Salmonella

A. After *Salmonella* samples have incubated in RV and TT broth tubes for 24 hours, transfer 50 µl aliquots of each RV sample enrichment to one XLD plate and one CHROMagar ™ plate per sample, and 50 µl aliquots of each TT sample enrichment to one XLD plate and one CHROMagar ™ plate per sample. Streak each plate for isolation with yellow loops.

B. Incubate the XLD and CHROMagar ™ plates at 35°C for 24 hours.

C. Store the RV and TT tubes at 4°C until final results are confirmed.

DAY 3

3.4 LISTERIA - Processing Positive results - Listeria (24 hour enrichment)

Note: MOX and LMPM plates from the 24 hour enrichment can be stored at 4°C (after 48 hours of incubation) and processed and evaluated with the 48 hour enrichment plates on Day 4.

A. Visually assess the MOX and LMPM plates and record bacterial growth levels on the paper copy of the Sample List (See Appendix B for a Sample List example).
   1. Total Bacterial growth (not just *Listeria*) will be assessed for each plate on the following scale:
      (a) "No" = no colony growth
      (b) "1 col" ("2 col" / "3 col" / etc.) = if < 5 colonies on the plate, record the #
      (c) "VL" = very light colony growth :approx. 5 – 60 colonies
      (d) "L" = light colony growth: approx.60-125 colonies
      (e) "M" = medium colony growth approx. 126-200 colonies
      (f) "H" = heavy colony growth or lawn approx. 200+

B. While recording bacterial growth, assess the MOX and LMPM plates for colonies that have Listeria like morphology.
   1. On MOX: "*Listeria* spp. colonies are approximately 2-3 mm diameter, black with a black halo and sunken center" – FDA BAM 2017. *Note: however, recent data indicate that some Listeria spp. may not have a sunken center.*
      (a) A silvery metallic shine can often be seen on *Listeria* colonies on MOX
   2. On LMPM: "*L. monocytogenes* (and *L. ivanovii*) produce a 1-3 mm diameter,

smooth, convex, blue/green colony and small blue/green halo. All other *Listeria*

species produce a 1-2 mm, smooth, convex white colony with no halo" – FDA BAM 2017

3. Refer to the positive control plates for morphology reference or consult a senior lab member for assistance with identification

C. Sub-streak putative positive results.

1. Sub-streak based on the following table onto either LMPM plate or BHI
   (a) Sub-streak onto LMPM if it is a mixed culture or are unsure if it is *Listeria.*
   (b) Sub-streak onto BHI if colony is already well isolated and confident that it is *Listeria.*
2. If possible sub streak colonies form the plates representing the 24 h enrichment. While positive results *may* be streaked without reading the 48 hour plate, the suggestion is to wait for the 48 h plate to be able to assess both the 24 and 48 h plates, unless it is very clearly *Listeria* or results are time sensitive.
3. If 24 hour plate has less than 4 colonies or not well isolated colonies, wait to sub-streak until viewing the 48 hour plate

Result	**Streaking Instruction**
Blue colonies on LMPM (indicating presence of *L. mono* or *L. ivanovii*) and either black colonies or no black colonies on MOX	Sub-streak 4 blue colonies from LMPM, no need to sub-streak from MOX (streak from 24 h plates unless there are <4 colonies on 24 h, in which case additional colonies from 48 h plate should

	be selected for a total of 4 colonies)
Blue or white colonies on LMPM (indicating presence of *L. mono* or *L ivanovii* (blue) and *L.* spp.) and either black colonies or no black colonies on MOX	Sub-streak 4 blue colonies from LMPM and 2 white colonies from LMPM, no need to sub-streak from MOX (streak from 24 h plates unless there are <4 blue colonies on 24 h or <2 white colonies, in which case additional colonies from 48 h plate should be selected for a total of 4 blue colonies and 2 white colonies.)
White colonies on LMPM (indicating presence of L. spp) and either black colonies or no black colonies on MOX	Sub-streak 2 white colonies from LMPM (streak from 24 h plates unless there are <2 colonies on 24 h, in which case additional colonies from 48 h plate should be selected for a total of 2 colonies) . No need to sub-streak from MOX
Black colonies on MOX (indicating presence of L. spp)	Sub-streak 4 black colonies from MOX (streak from 24 h plates

and neither white or blue colonies on LMPM	unless there are <4 colonies on 24 h, in which case additional colonies from 48 h plate should be selected for a total of 24 colonies)

4. Incubate the sub-streaked LMPM plates at 35°C and BHI plates at 37°C for 48 hours before assessing.

D. Negative plates can be put in BSL-1 waste. Controls and any plates associated with samples that have putative positives should be stored at 4°C until final results are confirmed.

E. Notify appropriate plant personnel of any putative positives via email.

3.5 SALMONELLA - Processing Positive Results – Salmonella

A. Visually assess the assess the XLD and CHROMagar™ plates and record total bacterial growth levels on the paper copy of the Sample List

1. Total bacterial growth (not just *Salmonella*) will be assessed for each plate on the following scale:

(a) "No" = no colony growth

(b) "1 col" ("2 col" / "3 col" / etc.) = if < 5 colonies on the plate, record the #

(c) "VL" = very light colony growth :approx. 5 – 60 colonies

(d) "L" = light colony growth: approx.60-125 colonies

(e) "M" = medium colony growth approx. 126-200 colonies

(f) "H" = heavy colony growth or lawn approx. 200+

B. While recording bacterial growth, assess the XLD and CHROMagar™ plates for colonies that have *Salmonella* like morphology.

1. On XLD: "[*Salmonella* appears as] pink colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies." – FDA BAM 2017
2. On CHROMagar™ *Salmonella* colonies typically appear purple/mauve in color. Other bacteria will appear colorless or blue.
3. Refer to the positive control plates for reference or consult a senior lab member for assistance with identification

C. Sub-streak putative positive results.

1. Sub-streak up to four (4) putative *Salmonella* colonies to XLD. 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.
2. Incubate the sub-streaked XLD plates at 35°C for 24 hours before assessing.

D. Negative plates can be put in BSL-1 waste. Controls and any plates associated with samples that have putative positives should be stored at 4°C until final results are confirmed.

E. Notify appropriate plant personnel of any putative positives via email.

DAY 4

3.6 LISTERIA - Processing Positive Results - Listeria (48 hour enrichment)

A. Repeat the steps described in 3.8 for the 48 hour enrichment plates (and 24 hour enrichment plates if they were stored at 4°C on Day 3).

Note: If dairy plant or other non-research samples are tested and if the 48 hour enrichment plating reveals a positive that was not found in the 24 hour enrichment plating, immediately notify appropriate plant personnel of the putative positive.

B. If colonies from the 24 hour enrichment plates were sub-streaked to LMPM on Day 3, follow the steps in 3.8 - Part B to visually assess the plates for the presences of *Listeria.*

1. If sub-streaks appear to be positive for *Listeria*, proceed with PCR verification described in Section 3.12.

C. If no positives are identified on both the 24 hour and 48 hour enrichment plates, proceed with reporting results as described in Section 3.13.

3.7 SALMONELLA - Processing Positive Results – Salmonella

A. Follow the steps in 3.9 - Part B to visually assess the sub-streaked plates for the presences of *Salmonella.*

1. If sub-streaks appear to be positive for *Salmonella*, proceed with sequencing verification as described in Section 3.12.
2. If all sub-streaks were rejected as positives, skip to reporting results as described in Section 3.13.

DAY 5+

3.8 Confirmation and Storage of Positive Results

A. Repeat the steps described in Section 3.8 – Part B to assess the LMPM plates sub-streaked on Day 4

1. If all samples and sub-streaks for both the 24 hour and 48 hour enrichment plates do not show evidence for presumptive Listeria or Salmonella positives, skip to reporting results as described in Section 3.13.

B. If sub-streaks plates from any time point appear to be positive for *Listeria* (or *Salmonella)*, sub-streak an isolated colony from that plate to BHI agar and incubate the BHI plates for 24h at 37°C (32°C for *Salmonella*). This plate should then be used for confirmation of the positive by either (i) *sigB* PCR with subsequent sequencing of the sigB PCR product (for Listeria spp.; see SOP 8.1.1.2.2.5 Sig B PCR Protocol located on the Food Safety Lab wiki); (ii) hly PCR for *L. monocytogenes;* (see SOP 8.1.1.2.2.3 hlyAB PCR Protocol located on the Food Safety Lab wiki*)* and (iii) *invA*

PCR for Salmonella (see SOP 8.1.1.3.6-InvA colony PCR for Salmonella located on the Food Safety Lab wiki)

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1. For *Listeria* isolates, compare the sequencing results to the Food Microbe Tracker database to assign a species.

2. For confirmed positives, select 4 colonies from the sub-streaked plates. Follow the SOP 2.5-FSL Isolate Designation, ID Assignment and Glycerol Stock Preparation on the Food Safety Lab wiki to assign these isolates FSL numbers and prepare them for cryogenic storage.
   (a) For Listeria positives, preference should be given to the colonies isolated from 24 hour enrichment plates (LMPM or MOX).
3. Add the new isolates to the Food Microbe Tracker database

3.9 Report final results

A. Record final sampling results in a Report Worksheet, indicating positive and negative results for each sample, including negative and positive controls.
   1. The Report Worksheet is prepared from the electronic copy of the Sample List. See Appendix B for a Report Worksheet example.
B. Attach the Report Worksheet to an email and forward to the appropriate plant personnel.
C. File the paper copy of the Sample List in the appropriate Processing Records binder.
   1. Also file a document containing the sample site, FSL # designation, species assignment (for *Listeria*), stained PCR gel image, and consensus sequence for all confirmed positives in the Processing Records binder.

APPENDIX A: MEDIA PREPARATION

Media for Listeria Isolation

A. Buffered Listeria Enrichment Broth (BLEB)

1. Suspend 48 g powder in 1 L of purified water
2. Ensure solids are completely dissolved. Use gentle heating if necessary
3. Autoclave at 121° C for 15 minutes.
4. Store at 4°C

B. Modified Oxford Agar (MOX)

1. Suspend 27.75 g of Oxford Agar Base in 500 ml of purified water. Mix well.
2. Ensure solids are completely dissolved. Use gentle heating if necessary
3. Autoclave at 121° C for 15 minutes
4. Cool to 55° C and aseptically add the contents of one vial of Modified Listeria Selective Supplement (Oxoid SR0206E).

C. Mix well and pour into sterile petri plates. Can be made on Master-Clave Auto Plate Pourer. MOX plates are light sensitive . (store in a dark place)

D. Listeria Selective Enrichment Supplement (50 ml)

Only prepare if the Oxoid SR0149A pre-made supplement is unavailable

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 180mg of Sodium Hydroxide (NaOH) in 15 ml sterile DH2O. 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 DH2O. 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 50ml using sterile DH2O. Protect completed mixture from light and store at 4°C.

E. *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 (store in a dark place).

Media for Salmonella Isolation

A. Lactose Broth (Difco)

1. Suspend 13 g powder in 1 Liter of purified water.
2. Ensure solids are completely dissolved. Use gentle heating if necessary
3. Autoclave at 121° C for 15 minutes.
4. Store at 4°C

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.

3. Store at 4°C. 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 9.0ml TT broth aseptically into 18 x 150 glass culture tubes. Broth can be stored at 2°C to 8°C for several weeks without the iodine solution.
2. Prepare Iodine Solution: In a fume hood, add 6 g Iodine and 5 g Potassium Iodide to 20 ml purified water in an amber/dark bottle. Vortex vigorously until the components are dissolved. Solid iodine will stain fabric and surfaces, use caution while handling. The iodine solution is light sensitive (store in a dark place). Store at 4°C.
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 L purified water.
2. Heat just to boiling. DO NOT AUTOCLAVE. Pour into sterile petri plates. XLD plates are light sensitive (store in a dark place) meaning they are stored in a dark place in the cold room.

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 between 45° C to 50°C. Pour into sterile petri plates. CHROMagar™

Salmonella plates are light sensitive (store in a dark place).

APPENDIX B: REPORT EXAMPLES

Sample List Examples:

Cornell Dairy Environmental Sampling Report

During Production Plant Swabbing #86-75309

Sample Date: 4/24/91 Sampler name: Milkayla Caseindy

Report Date:

Monthly Rotation	Zone	Site ID	Brief Sample Name	Description of sample site	Sampling instructions	24 HR MOX	24 HR LMPM	48 HR MOX	48 HR LMPM
Rotate 1-5, note in remarks	2	1a	FV3	Flavor Vat 3 lid	Swab exterior of tank lid 2'x2' area	VL	VL	L	M
	2	2a	PT3	Pasteurized Tank 3	Swab exterior of door of tank 2'x2' area				
	2	3a	Gallon Filler	Gallon Bowl	swab exterior of bowl 2'x2' area	no	VL	no	M
	2	4a	8oz.	8oz. Bowl	swab exterior of bowl 2'x2' area				
	2	5a	Yogurt	Yogurt Machine Bowl	swab exterior of bowl 2'x2' area	M	M	H	H
Rotate 6-10, note in remarks	2	6	Fruit Feeder	Machine Controls	swab control buttons associated with machine	3 col	VL	VL	no

Cornell Dairy Environmental Sampling Report

During Production Plant Swabbing #86-75309

Sample Date: 4/24/91 Sampler name: Milkayla Caseindy

Report Date:

Monthly Rotation	Zone	Site ID	Brief Sample Name	Description of sample site	Sampling instructions	TT - XLD	TT - CHROMagar	RV - XLD	RV - CHROMagar
Rotate 1-5, note in remarks	2	1a	FV3	Flavor Vat 3 lid	Swab exterior of tank lid 2'x2' area	VL	VL	L	M
	2	2a	PT3	Pasteurized Tank 3	Swab exterior of door of tank 2'x2' area				
	2	3a	Gallon Filler	Gallon Bowl	swab exterior of bowl 2'x2' area	no	VL	no	M
	2	4a	8oz.	8oz. Bowl	swab exterior of bowl 2'x2' area				
	2	5a	Yogurt	Yogurt Machine Bowl	swab exterior of bowl 2'x2' area	M	no	H	L
Rotate 6-10, note in remarks	2	6	Fruit Feeder	Machine Controls	swab control buttons associated with machine	1 col	no	VL	no

Report Worksheet Examples:

Environmental Sampling Report

During Production Plant Swabbing #86-75309

Sample Date: 4/24/91
Report Date: 7/20/91

Sampler name: Milkayla Caseindy, QC Manager

Monthly Rotation	Zone	Site ID	Brief Sample Name	Description of sample site	Sampling instructions	L.mono	L.species
Rotate 1-5, note in remarks	2	1a	FV3	Flavor Vat 3 lid	Swab exterior of tank lid 2'x2' area	positive	neg
	2	2a	PT3	Pasteurized Tank 3	Swab exterior of door of tank 2'x2' area		
	2	3a	Gallon Filler	Gallon Bowl	swab exterior of bowl 2'x2' area	neg	neg
	2	4a	8oz.	8oz. Bowl	swab exterior of bowl 2'x2' area	neg	positive - L. innocua
	2	5a	Yogurt	Yogurt Machine Bowl	swab exterior of bowl 2'x2' area		
Rotate 6-10, note in remarks	2	6	Fruit Feeder	Machine Controls	swab control buttons associated with machine		
	2	7	Gallon Filler	Machine Controls	swab control buttons associated with machine	neg	neg
	2	8	8oz. Filler	Machine Controls	swab control buttons associated with machine		

Environmental Sampling Report

During Production Plant Swabbing #86-75309

Sample Date: 4/24/91
Report Date: 7/20/91

Sampler name: Milkayla Caseindy, QC Manager

Monthly Rotation	Zone	Site ID	Brief Sample Name	Description of sample site	Sampling instructions	Salmonella
Rotate 1-5, note in remarks	2	1a	FV3	Flavor Vat 3 lid	Swab exterior of tank lid 2'x2' area	positive
	2	2a	PT3	Pasteurized Tank 3	Swab exterior of door of tank 2'x2' area	
	2	3a	Gallon Filler	Gallon Bowl	swab exterior of bowl 2'x2' area	neg
	2	4a	8oz.	8oz. Bowl	swab exterior of bowl 2'x2' area	neg
	2	5a	Yogurt	Yogurt Machine Bowl	swab exterior of bowl 2'x2' area	
Rotate 6-10, note in remarks	2	6	Fruit Feeder	Machine Controls	swab control buttons associated with machine	
	2	7	Gallon Filler	Machine Controls	swab control buttons associated with machine	
	2	8	8oz. Filler	Machine Controls	swab control buttons associated with machine	

SECTION 4 TROUBLESHOOTING

4.1 Contamination with the *Listeria* positive control.

If a *Listeria monocytogenes* positive is identified in a sample, there is a chance it is not a true positive and was contaminated by the positive control. If this is suspected, the Δ*actA* isolate used as the positive control can be differentiated from a true environmental *Listeria* positive by following the SOP 8.1.1.2.2.1-actA PCR on the Food Safety Lab wiki.

SECTION 5 REFERENCES

U.S. Food and Drug Administration (USFDA). *Bacteriological Analytical Manual.* 2017. Accessed online: 04/29/2020. https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

SECTION 6 METHOD VERSION & CHANGES

VERSION	DATE	EDITOR	COMMENTS
Version 1	01/29/2013	Sarah Beno	Original SOP: (Update from unknown prior document adopted from the FDA BAM protocol)
Version 2	04/29/2020	Jordan Skeens	Updated to 04/2020 SOP template Changed reference to L.mono 10403S positive control to the Δ*actA* L.mono mutant R3-0001. Title page: updated the SOP name in FILE NAME and document headers Section 1.2: Updated the scope to include all potential users Section 1.3: Added safety info Section 2: Edited the formatting of the Materials list, and added sterile sponge bags, dH_2O, and yellow loops Section 3 & 4: Section 4 was merged into Section 3, former Section 3 info moved to newly created Appendix A Section 3: (Formerly Section 4) Significantly expanded and edited the procedures to add important details, remove obsolete info and streamline the workflow. Section 3 – Appendix B: Created with examples of the reports Section 4: (Formerly Section 5) Added direction to use *actA* PCR for *Listeria* positive control contamination verification Section 5: (Formerly Section 6) Added a reference for the FDA BAM
Version 3	03/05/2021	Jordan Skeens	Section 3.12: Added that putative positives need to be Sub-streaked to BHI for PCR sequencing confirmation
Version 4	3/08/23	Caroline Motzer	Section 3.1 A: added note about Cornell Dairy sampling Section 3.2. B. Added to grab only 1.3-1/2 of colony, Added to store the negative next to the control and that ideally the positive control is inoculated with about 100 Cfu of listeria Section 3.31A: Changed 225 ml of lactose broth to 90 ml Section 3.3B: Changed 225 ml of lactose broth to 90 ml add that negative and positive should be stored together

			Section 3.4A: added numbers for descriptors Section 3.4 B: some listeria may not have a sunken center Section 3.4 C: added chart of how to streak Section 3.5 A: added numbers for descriptors Section 3.6: added note to notify plant personal of presumptive positive Section 3.8 B: hyperlinked appropriate SOPs Appendix A, A and B: added step to ensure dissolving of contents, use heating if necessary