Assurance® *GDS*
Listeria monocytogenes Tq

There are two validated methods that can be followed:

- AOAC® Performance Tested Method 070702
- Health Canada Method MFLP-07

Part No: 71010-100 (100 tests)
71010-576 (576 tests)
71010-576 ATM (576 tests)

General Description

Assurance® GDS, genetic detection system, for *Listeria monocytogenes* Tq is an automated nucleic acid amplification system for the detection of *Listeria monocytogenes* from a variety of foods and surfaces including ready-to-eat meat, seafood, dairy products, produce, pasteurized soft cheese, stainless steel, rubber, plastic and concrete surfaces.

Kit Components

Each Assurance GDS *Listeria monocytogenes* Tq kit contains the following:

- Amplification Tubes Tq
- Listeria Concentration Reagent
- Listeria Resuspension Buffer Tq
- Wash Solution

Each Assurance GDS for *Listeria monocytogenes* Tq 576ATM kit contains the following:

- Amplification Tubes Tq
- Concentration Reagent

The following are also necessary but sold separately:

- 61031-100 Wash Solution Kit
- 34745-100C *Listeria* Resuspension Buffer Tq

Equipment / Materials Required

Other necessary materials not provided include:

- Enrichment media (see Appendix A)
- Assurance GDS Rotor-Gene®
- PickPen® and PickPen tips
- Vortex mixer
- Adhesive film
- Sample wells and sample wells base
- Resuspension Plate
- Gel Cooling Block
- 8-channel micropipette capable of dispensing 30 µL
- Repeat pipette

Repeat pipette tips (0.5 mL and 10 mL) Adjustable micropipette

Filter barrier micropipette tips (50 µL and 1.0 mL)

Stomacher / Masticator or equivalent

Incubators capable of maintaining 35 – 37 °C and 30 °C

Additional materials for the 576 kit include:

Variable Spacing Multi-Channel Pipette

Aluminum Cooling Block, 72 well

72-well rotor and locking ring

AOAC® PERFORMANCE TESTED METHOD 070702

Approved matrices include: Sliced Deli Meat, Hot Dogs, Raw Fish, Raw Green Beans, Liquid Milk, Pasteurized Soft Cheese and Environmental Surfaces

Sample Preparation

A. Test Portion Preparation & Enrichment

a. Add 25 g of sample to 225 mL of the appropriate enrichment media (Appendix A).

Note: Demi-Fraser media must be pre-warmed per instructions in Appendix.

b. Collect environmental surface samples with a sponge or swab hydrated with D/E (Dey/Engley) Broth or Letheen Broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of the appropriate enrichment media respectively (Appendix A).

Note: Sponges and swabs hydrated with Neutralizing Buffer are not recommended for use with Assurance GDS for *Listeria monocytogenes.*

c. **22 hour enrichment protocol** - Incubate samples for 18 – 24 h at 35 – 37 °C.

d. **30 hour enrichment protocol** - Incubate samples for 30 – 48 h at 30 °C.

B. Sample Preparation Protocol

Change gloves prior to handling Reagents

22 hour Protocol

a. Transfer 1.0 mL of incubated sample to empty wells in sample block (1 well / sample) and cover sample wells with adhesive film strips. Avoid transferring food particles. Incubate for 4 – 8 h at 30 °C.

b. Vortex **Sample Concentration Reagent.** Immediately transfer 20 µL to each of the wells containing incubated sample using a repeat pipette and 0.5 mL pipette tips. Cover sample wells with adhesive film strips. Proceed directly to step (e).

30 hour Protocol

c. Vortex **Sample Concentration Reagent.** Immediately transfer 20 µL to each of the required number of empty Assurance GDS sample wells (1 well / sample) using a repeat pipette and 0.5 mL pipette tips. Cover sample wells with adhesive film strips.

d. Add 1.0 mL of incubated sample to each sample well containing Sample Concentration Reagent. Avoid transferring food particles. Cover sample wells with adhesive film strips. Proceed directly to step (e).

22 and 30 hour Protocols

e. Place sealed sample wells containing Sample Concentration Reagent and sample on the vortex mixer and vortex at 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

f. Transfer 1.0 mL of **Wash Solution** to additional wells (1 well / sample) using a repeat pipette and 10 mL pipette tips. Cover sample wells with adhesive film strips.

g. Add 45 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover prepared resuspension plate with adhesive film strips.

h. Carefully remove and discard adhesive film strip from a strip of samples.

i. Load tips onto the PickPen®, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Tap the PickPen tips against the side of the sample wells to remove excess media droplets.

j. Remove adhesive film strip from the corresponding wells containing Wash Solution. Transfer PickPen to the Wash Solution. With tips submerged, gently stir the PickPen from side to side for 5 – 10 s. Tap the PickPen tips against the side of the sample wells to remove excess wash solution droplets.

k. Remove adhesive film strip from resuspension plate. Transfer PickPen to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq.

l. Repeat steps (h) through (k) for all samples using new tips for each strip of samples.

m. Cover resuspension plate with adhesive film strips.

n. Place sealed resuspension plate containing samples in 60 – 64 °C incubator for 15 min – 1 h.

PROCEED TO TEST PROCEDURE

HEALTH CANADA METHOD MFLP-07

Approved matrices include: Raw Fermented Sausage, Sliced Deli Meat, Hot Dogs, Liver Pate, Raw Fish, Cooked Fish, Frozen Fish, Raw Spinach, Raw Green Beans, Pasteurized Soft Cheese, Liquid Milk and Environmental Surfaces.

Sample Preparation

A. Test Portion Preparation & Enrichment

a. For foods, add 25 g of sample to 225 mL of the appropriate enrichment media (Appendix A). Masticate sample with the media to mix well.

b. For environmental monitoring, collect environmental surface samples with a sponge or swab hydrated with D/E (Dey/Engley) Broth or Letheen Broth. After collecting sample, add sponge or swab to 100 mL or 10 mL of the appropriate enrichment media respectively (Appendix A). Masticate sponge and media to mix well.

Note: Sponges and swabs hydrated with Neutralizing Buffer are not recommended for use with Assurance GDS for *Listeria monocytogenes.*

c. **22 hour enrichment protocol** - Incubate samples for 18 – 24 h at 35 – 37 °C. Transfer 1.0 mL to sample block wells containing Concentration Reagent and incubate for 4 – 8 h at 30 °C per step B (d) below.

d. **Raw and cooked seafood protocol** - Incubate samples for 18 – 24 h at 35 - 37 °C. Transfer 1.0 mL to empty sample wells and incubate for 4 – 8 h at 30 °C per step B (d) below.

e. **Frozen seafood –** Incubate samples for 22 – 26 h at 30 °C and transfer to 0.5 mL of fresh Demi Fraser Broth and incubate for 4 – 6 h at 30 °C per step B (e) below.

f. **30 hour enrichment protocol** - Incubate samples for 30 – 48 h at 30 °C.

g. **36 hour enrichment protocol** – incubate samples for 36 – 48 h at 30 °C.

B. Sample Preparation Protocol

Change gloves prior to handling Reagents

a. For all protocols except raw and cooked seafood protocol, vortex **Sample Concentration Reagent.** Immediately transfer 20 µL to each of the required number of Assurance GDS sample wells (1 well / sample) using a repeat pipette and 0.5 mL pipette tips. Cover sample wells with adhesive film strips.

b. For all protocols Transfer 1.0 mL of **Wash Solution** to additional sample wells (1 well / sample) using a repeat pipette and 10 mL pipette tips. Cover sample wells with adhesive film strips.

c. For frozen seafood, transfer 1.0 mL of Wash Solution to a second set of empty sample wells and transfer 0.5 mL of sterile Demi Fraser Broth to another set of sample wells (1 well / sample).

d. For all protocols except raw and cooked seafood, add 1.0 mL of incubated sample to each sample well containing Sample Concentration Reagent. For raw and Cooked Seafood transfer 1.0 mL aliquot to empty sample well). Avoid transferring food particles. A new pipette tip must be used for each sample. Cover each strip of sample wells with a new adhesive film strip prior to adding samples to a new strip.

   30 hour protocol - proceed directly to step (e).

   36 hour protocol - proceed directly to step (e).

   22 hour protocol - Incubate sealed sample wells containing 1.0 mL of sample for 4 – 8 h at 30 °C.

   Raw and cooked seafood protocol – incubate sealed sample wells containing 1.0 mL of sample for 4 – 8 h at 30 °C. Following incubation, Vortex **Sample Concentration Reagent.** Immediately transfer 20 µL to each sample well containing 1 mL of enriched sample using a repeat pipette and 0.5 mL pipette tips. Cover sample wells with adhesive film strips.
   Frozen seafood protocol - proceed directly to step (e).

e. Place sealed sample wells containing Sample Concentration Reagent and sample on the vortex mixer and vortex at 900 rpm for 10 – 20 min. If necessary, adjust rpm to be certain that liquid does not contact adhesive film.

f. For frozen seafood protocol only,

   a) Carefully remove and discard adhesive film strip from a strip of samples.

   b) Load tips onto the PickPen, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 s while continually moving up and down from the surface to the bottom of the well. Tap the PickPen tips against the side of the sample wells to remove excess media droplets.

   c) Remove adhesive film strip from the corresponding wells containing Wash Solution. Transfer PickPen to the Wash Solution. With tips submerged, gently stir the PickPen from side to side for 5 – 10 s. Tap the PickPen tips against the side of the sample wells to remove excess wash solution droplets.

   d) Remove adhesive film strip from corresponding wells containing 0.5 mL of fresh Demi Fraser Broth. Transfer PickPen to corresponding row of Demi Fraser Broth sample wells. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Demi Fraser Broth

   e) Cover wells containing Demi Fraser Broth and Concentration Reagent particles and incubate for 4 – 6 h @ 30 °C. Proceed directly to step (g).

g. For all protocols, add 45 µL of **Resuspension Buffer Tq** to the sample wells in the resuspension plate using a repeat pipette and a 0.5 mL pipette tip. Cover prepared resuspension plate with adhesive film strips.

h. Carefully remove and discard adhesive film strip from a strip of samples.

i. Load tips onto the PickPen, ensuring that the tips are firmly in place on the PickPen tool. Extend the PickPen magnets and insert into the first strip of sample wells. Stir gently for 30 sec while continually moving up and down from the surface to the bottom of the well. Tap the PickPen tips against the side of the sample wells to remove excess media droplets.

j. Remove adhesive film strip from the corresponding wells containing Wash Solution. Transfer PickPen to the Wash Solution. With tips submerged, gently stir the PickPen from side to side for 5 – 10 s. Tap the PickPen tips against the side of the sample wells to remove excess wash solution droplets.

k. Remove adhesive film strip from resuspension plate. Transfer PickPen to the corresponding row of the prepared resuspension plate. With tips submerged, retract the PickPen magnets and tap gently to release particles into the Resuspension Buffer Tq. Cover wells with adhesive film strip.

l. Repeat steps (g) through (j) for all samples using new tips for each strip of samples.

m. Place sealed resuspension plate containing samples in 60 °C incubator for 15 min – 1 h.

Test Procedure

A. Preparation of Gel Cooling Block

a. Prior to initial use, the gel cooling block must be stored in the freezer (-25 to -15 °C) for 6 h. When frozen the gel cooling block will change color from pink to purple. When not in use the gel cooling block should continue to be stored at -25 to -15 °C.

b. Between each use the gel cooling block should be returned to the freezer until it has turned completely purple, indicating it is ready for use. This may take up to 2 h.

c. The aluminum cooling block is for use with the 576 test kit and should be stored in the refrigerator (2 – 8 °C). To use, place the refrigerated aluminum cooling block on top of the frozen gel cooling block.

B. Preparation of Amplification Tubes

a. The Assurance GDS Rotor-Gene set up and data entry should be completed prior to transferring samples from the resuspension plate into the Amplification Tubes.

b. Remove **Amplification Tubes Tq** from foil pouch and place them in the frozen gel cooling block (aluminum cooling block for 576 test kit). Reseal pouch.

c. Transfer 30 µL of sample from the resuspension plate wells into each Amplification Tube using a multi-channel pipette and filter barrier tips. Firmly press down on each Amplification Tube lid to close. Visually inspect each tube to ensure that the cap is securely sealed.

d. Place Amplification Tubes into Assurance Rotor-Gene in sequential order, beginning with position #1. For the 100 test kit, use the 36-well rotor and locking ring; for the 576 test kit, use the 72-well rotor and locking ring.

Note: For 576 test kit, after loading amplification tubes in the rotor and securing with locking ring, contents should be thoroughly mixed by shaking with a snapping motion. See Application Note FRMMK.2060 for details.

e. Start Rotor-Gene cycle. Refer to Assurance GDS user manual for detailed instructions on operating the Rotor-Gene.

Note: The Assurance GDS Rotor-Gene must be started within 20 min after addition of the samples to the Amplification Tubes.

Results

Upon completion of the run, the Assurance GDS Rotor-Gene software will provide a results table. Each sample will be identified as **Positive**, **Negative**, or **No Amp.**

No.	Color	Name	Result	Description	Kit Lot Number
1		Sample 1	Positive	*L. monocytogenes*	1234567
2		Sample 2	Negative	*L. monocytogenes*	1234567
3		Sample 3	No Amp	*L. monocytogenes*	1234567

Positive: Samples are positive for *L. monocytogenes*

Negative: Samples are negative for *L. monocytogenes*

No Amp: Amplification did not occur. Repeat the test beginning from Step B. Sample Preparation Protocol. If the No Amp result repeats, contact BioControl Technical Service.

Confirmation

AOAC PTM 070702:

Samples can be confirmed via the following:

a. U.S. Department of Agriculture (USDA) *Microbiology Laboratory Guidebook* (http://www.fsis.usda.gov)

b. U.S. Food and Drug Administration *Bacteriological Analytical Manual* (http://www.cfsan.fda.gov)

MFLP-07:

Presumptive positive results may be confirmed from the primary enrichment broth by proceeding with the plating and confirmation steps of an appropriate reference method such as MFHPB-07 or MFHBP-30.

Storage

Store Assurance GDS for *Listeria monocytogenes* Tq kit components at 2 – 8 °C. Kit expiration is provided on the product box label.

Precautions

If possible, maintain separate work zones and dedicated equipment and supplies for sample preparation and amplification and detection.

It is recommended to utilize both positive and negative control samples.

This product is not intended for human or veterinary use. Assurance GDS for *Listeria monocytogenes* Tq must be used as described herein. Contents of the test may be harmful if swallowed or taken internally.

Do not use test kit beyond expiration date on the product box label. Decontaminate and dispose of materials in accordance with good laboratory practices and in accordance with local, state and federal regulations.

Do not open or autoclave used Amplification Tubes. After run is complete, place used Amplification Tubes into a sealed container with sufficient volume of a 10% bleach solution to cover tubes for a minimum of 15 min or double bag amplification tubes and dispose outside of the lab.

If contamination is suspected, moisten paper towel with bleach solution and wipe all lab benches and equipment surfaces with 10% bleach solution. Avoid spraying bleach solution directly onto surfaces. Allow bleach solution to remain on surfaces for a minimum of 15 min before wiping clean with 70% isopropyl alcohol solution.

To prepare 10% bleach solution add 10 mL of commercially available bleach containing at least 5% sodium hypochlorite to 90 mL of deionized water. The minimum final concentration of sodium hypochlorite in the bleach solution should be 0.5%. The bleach solution is stable for 7 days from preparation. To prepare 70% isopropyl alcohol solution add 70 mL of pure isopropyl alcohol to 30 mL of deionized water or buy commercially available 70% isopropyl alcohol.

Waste may be contaminated with *Listeria* which is potentially hazardous to human health. All biohazard waste should be disposed of appropriately.

Pregnant women, elderly, and potentially immune-compromised individuals must be prohibited from laboratory rooms or areas where *Listeria monocytogenes* enrichment, isolation, and identification procedures are in progress. Although a properly sanitized laboratory area should not harbor *Listeria*, supervisors should use their own discretion in allowing these high-risk individuals into these areas.

Appendix A – Enrichment Protocols and Media

AOAC PTM 070702 Sample Type	Enrichment Protocol	Enrichment Media
Fish & Seafood	22 hour	BLEB + LiCl + PALCAM
Fruits & Vegetables	22 hour	BLEB + LiCl + PALCAM
Dairy Products Pasteurized Soft Cheese	22 hour 30 hour	BLEB + LiCl + PALCAM Demi-Fraser Broth
Meat & Poultry Hot dogs	30 hour 22 hour	Demi-Fraser Broth BLEB + LiCl + PALCAM
Environmental	22 hour	BLEB + LiCl + PALCAM
Other Foods	30 hour	Demi-Fraser Broth

MFLP-07 Sample Type	Enrichment Protocol	Enrichment Media
Raw or Cooked Fish & Seafood	Raw and Cooked Seafood Protocol	BLEB + LiCl + PALCAM
Frozen Fish & Seafood	Frozen Seafood protocol	Demi Fraser Broth
Dairy Products except Soft Cheese	22 hour	BLEB + LiCl + PALCAM
Pasteurized Soft Cheese	30 hour	Demi-Fraser Broth
Spinach	36 hour	Demi-Fraser Broth
RTE Meats	36 hour	Demi-Fraser Broth
Environmental	22 hour 36 hour	BLEB + LiCl + PALCAM Demi-Fraser Broth

Buffered Listeria Enrichment Broth with Lithium Chloride and PALCAM Antimicrobic Supplement (BLEB + LiCl + PALCAM)

Suspend 48 g of dehydrated Buffered Listeria Enrichment Broth (BLEB) Base in 1.0 L of purified water. Add 5 g Lithium Chloride (LiCl). Mix thoroughly, dispense into desired aliquots and autoclave at 121 °C for 15 min.

Alternatively, add 3.4 mL of sterile 8M LiCl solution to

225 mL of sterile BLEB Base media.

Aseptically add 10 mL sterile purified water to PALCAM Antimicrobic supplement powder and shake to dissolve.

On day of use, aseptically add the appropriate volume of PALCAM Antimicrobic supplement to BLEB + LiCl at a ratio of 225 µL supplement per 1.0 L BLEB (i.e. 25 µL supplement into 100 mL BLEB or 55 µL supplement into 225 mL BLEB).

Demi-Fraser Broth Base

Suspend 55 g of dehydrated Demi-Fraser Broth Base in 1.0 L of purified water. Mix thoroughly, dispense into desired aliquots and autoclave at 121 °C for 15 min. Pre-warm to
35 – 37 °C prior to use.

Buffered Listeria Enrichment Broth Base

Formula per Liter	
Pancreatic digest of casein	17.0 g
Soy peptone	3.0 g
Dextrose	2.5 g
Sodium chloride	5.0 g
Dipotassium phosphate	2.5 g
Disodium phosphate	9.6 g
Monopotassium phosphate	1.35 g
Yeast extract	6.0 g
Sodium pyruvate	1.1 g

PALCAM Antimicrobic Supplement

Formula per 10 mL	
Ceftazidime	40.0 mg

Demi-Fraser Broth Base

Formula per Liter	
Tryptose	10.0 g
Beef extract	5.0 g
Yeast extract	5.0 g
Sodium chloride	20.0 g
Disodium phosphate	9.6 g
Monopotassium phosphate	1.35 g
Esculin	1.0 g
Nalidixic Acid	0.01 g
Acriflavine HCl	12.5 mg
Lithium Chloride	3.0 g

Manufacturing Entity

BioControl Systems, Inc, 12822 SE 32nd St, Bellevue, WA 98005, USA.
BioControl Systems, Inc is an affiliate of Merck KGaA, Darmstadt, Germany.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803
United States

Lit.No.: MS_UG6412EN
03/2020