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RESEARCH INSTITUTE

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ASSAY PRINCIPLES

Veriflow® *Listeria monocytogenes* (LM) is a molecular based assay for the presumptive and qualitative detection of *Listeria monocytogenes*. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes post PCR amplification and requires only 24 hours of incubation for maximum sensitivity. The Veriflow® LM system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow® LM provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER

The Veriflow® LM system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with foodborne pathogen detection. Veriflow® LM is specifically designed to be easy-to-use and eliminates the need for advanced training in molecular biology.

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MATERIALS PROVIDED

1. IS *LM* PCR Reagent - Cat. No. IS0502
2. IS BUFFER B - Cat. No. IS0702
3. IS Veriflow® Assay Cassette - Cat. No. IS0101LM
4. 1.5 ml Sampling Tubes - Cat. No. IS0935
5. IS *Listeria* Broth - Cat. No. IS0302

MATERIALS NEEDED for ENVIRONMENTAL SAMPLES

1. Dry or pre-moistened sampling sponges OR swabs
 - a. Sponge should not exceed 10 ml, and Swab should not exceed 2 ml of hydration with Dey-Engley broth.
2. Dey-Engley broth (if necessary)
3. 18 oz. (532 ml) incubation bag for sponge samples
4. 2 oz. (58 ml) incubation bag for swab samples

MATERIALS NEEDED for READY-TO-EAT (RTE) SAMPLES

1. 24 oz. (710 ml) Incubation Bags (for Hot Dog and Milk Samples)
2. 55 oz. (1626 ml) Incubation Bags (for Deli Turkey Meat Samples)

MATERIALS NEEDED for ALL ENRICHMENT TYPES

1. Incubator that provides continuous and stable temperatures of $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$
2. Water bath for boiling or heating block ($95^{\circ}\text{C} \pm 2^{\circ}\text{C}$)
3. Heat tolerant beaker (optional for boiling)
4. PCR Thermocycler
5. Pipettes and tips for 5, 200 and 1000 μl volumes
6. Glassware and autoclave for media prep
7. Racks for culture bags and 1.5 ml tubes
8. dH_2O
9. Scale for weighing of sample and media

STORAGE OF MATERIALS

The Veriflow® *LM* kit components, including cassettes, plastics, growth media and buffers should be stored at room temperature ($20\text{-}25^{\circ}\text{C}$). The Veriflow® *LM* test PCR reagents should be stored at $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Store autoclaved liquid media in the dark (photosensitive) at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for a maximum of 30 days.

PRECAUTIONS

1. *Listeria monocytogenes* is a human pathogen. All samples collected for use with the Veriflow® *LM* Assay should be handled with care.
2. Assay users should observe standard microbiological practices and safety precautions when performing this assay.
3. Do not use Veriflow® *LM* Assay cassettes past indicated expiration date.
4. Do not use IS *Listeria* Broth past indicated expiration date.
5. Use rehydrated IS *Listeria* Broth within 30 days of preparation.
6. Deviations from the assay protocol may impact overall test performance.

NOTE: See Appendix 1 for matrices tested

For environmental samples, go to section I and II.

For ready-to-eat samples, go to sections III and IV.

For dairy samples, go to section V.

MEDIA PREP

1. Add 39 grams IS *Listeria* Broth media per 1 Liter dH₂O and autoclave for 15 minutes at 121°C (Media is photosensitive and can be stored at 4°C in the dark for a maximum of 30 days).
2. Allow media to equilibrate to room temperature (20-25°C) before use.

SECTION I: SPONGE SURFACE SAMPLING AND ENRICHMENT (Swabs: see Section II)

1. If sponges are not pre-moistened, pipette 10 ml of Dey-Engley Neutralization broth to each sponge placed in an 18 oz (532 ml) incubation bag.
2. Squeeze excess moisture from sponge and remove from incubation bag.
3. Sample area of interest on stainless steel surface using sponge for 30 seconds.
4. Transfer sponge back to incubation bag.
5. Transfer 100 ml IS *Listeria* Broth into bag containing sponge used for sampling, seal, and agitate sponge for 30 seconds.
6. Place bag into 35°C ± 2°C incubator, in rack, for 24-28 hours.

SECTION II: SWABS SURFACE SAMPLING AND ENRICHMENT (Sponges: see Section I)

1. If swabs are not pre-moistened, pipette 2 ml of Dey-Engley Neutralization broth to each swab placed in a 2 oz (58 ml) incubation bag.
2. Squeeze excess moisture from swab and remove from incubation bag.
3. Sample surface area of interest using swab for 30 seconds.
4. Transfer swab back to incubation bag.
5. Transfer 20 ml IS *Listeria* Broth into bag containing swab used for sampling, seal, and agitate swab for 30 seconds.
6. Place bag into 35°C ± 2°C incubator, in rack, for 24-48 hours.

SECTION III: SAMPLING AND ENRICHMENT for 25 GRAM RTE SAMPLE

1. Weigh out 25 gram sample.
 - a. Optional: if pre-packaged, spray and wipe package with 70% isopropanol to sanitize before removing sample
2. Transfer 25 gram sample to 24 oz. incubation bag.
3. Transfer 225 ml media prepared above to incubation bag from step 2.
4. Stomach 30 seconds to break down sample.
5. Place bag into 35°C ± 2°C incubator, in rack, for 24-28 hours.

SECTION IV: SAMPLING AND ENRICHMENT for 125 GRAM DELI MEAT SAMPLES

1. Weigh out 125 gram Deli Meat sample.
2. Transfer 125 gram sample of Deli Meat to 55 oz. (1626 ml) incubation bag.
3. Transfer 375 ml media prepared above to incubation bag from step 2.
4. Stomach 30 seconds to break down sample.
5. Place bag into 35°C ± 2°C incubator, in rack, for 24-28 hours.

SECTION V: SAMPLING AND ENRICHMENT for DAIRY SAMPLES

1. Transfer 225 ml media prepared above to 24 oz. (710 ml) incubation bag.
2. Transfer 25 ml or 25 g sample directly to bag from step 1.
3. Agitate bag to evenly distribute sample.
4. Place bag into 35°C ± 2°C incubator, in rack, for 24-28 hours.

SAMPLE PREP and PCR

1. Place provided 1.5 ml sampling tubes in rack (1 for each sample to be tested).
2. Remove incubation bag from incubator and agitate to suspend any settled contents.
3. Pipette 500 µl of enriched culture to prepared 1.5 ml tube from step 1 above, seal and invert to mix contents.

4. Boil 1.5 ml tube with sample in water bath or heating block for 10 ± 1 minutes and allow to cool for at least 10 minutes at room temperature (20-25°C).
 - a. Note: samples can be stored sealed at $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$, pre or post boil, for 1 week, prior to step 5 below.
5. Transfer 5 μl of liquid from cooled sample from step 4 above to thawed PCR reagent tube for each sample (thaw PCR tube for 10 ± 1 minutes at room temperature [20-25°C] and use immediately).
 - a. Note: Open PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
6. Cycle sample PCR tube in thermocycler with VFLOWLM program.

FLOWTHROUGH CASSETTE SAMPLE ANALYSIS

1. Remove tubes from thermocycler and add 4 drops of BUFFER B directly to each PCR tube.
2. Transfer entire contents (200 μl) of PCR tube directly to Veriflow[®] LM Assay cassette sample window with pipette. A separate Veriflow[®] LM Assay cassette must be used for each PCR tube.
3. Allow test to develop for 2 minutes \pm 15 seconds.
4. Add 4 drops of BUFFER B directly to each Veriflow[®] LM Assay cassette sample window.
5. Allow test to develop for 1 minutes \pm 15 seconds.
 - a. Optional: test may develop for up to 120 minutes before retraction and recording of results.
6. Retract switch and immediately record results.
 - a. The appearance of one red line (control) in the cassette window indicates a negative result.
 - b. The appearance of two red lines (control and test) in the cassette window indicates a positive result.
 - c. If the control line fails to develop, the test is invalid and will need to be repeated.

CUSTOMER SERVICE

Invisible Sentinel customer service and technical assistance can be reached between 9AM and 5PM Eastern time by calling 215-966-6118 and asking for an Invisible Sentinel sales or technical representative. Training on this product and all Invisible Sentinel test kits is available.

MSDS INFORMATION AVAILABLE

Material Safety Data Sheets (MSDS) are available for this test kit and all of Invisible Sentinel's Food test kits by calling Invisible Sentinel at 215-966-6118.

APPENDIX 1: MATRICES TESTED

Surfaces:

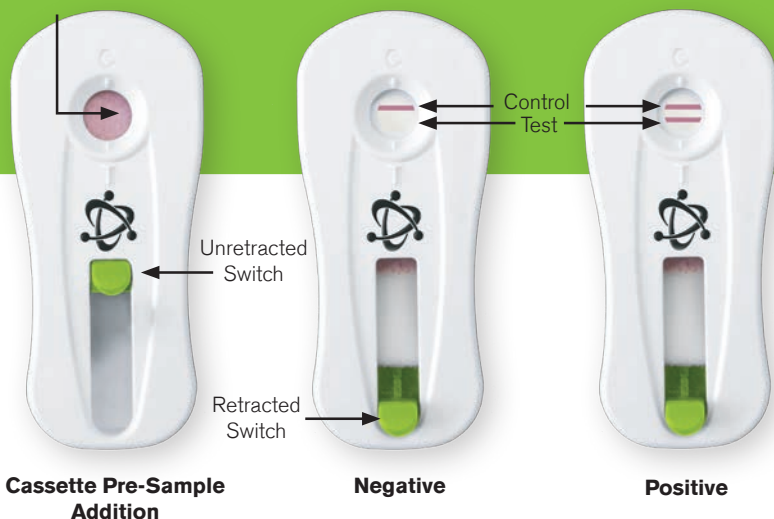
Stainless Steel
Sealed Concrete
Plastic
Ceramic Tile

Food (RTE) & Beverages (Dairy):

Deli Turkey Meat
Hot Dogs
2% milk

APPENDIX 2: RESULTS INTERPRETATION

Sample Window (Conjugate Pad)



The control line, as indicated by the letter C on the test cassette, should always develop. The test line, as indicated by the letter T on the cassette, will only develop in the event of a positive sample for *Listeria monocytogenes*. If the control line fails to develop, the test is invalid, and will need to be repeated.

APPENDIX 3: CONFIRMATION OF RESULTS

Presumptive positive samples must be confirmed by the USDA/FSIS or AOAC method for the detection of *Listeria monocytogenes* from environmental samples and food matrices.

Enriched samples (un-boiled) from the sampling swabs achieved using this protocol can be used for the confirmation, following the necessary steps laid out in the USDA/FSIS Microbiology Laboratory Guidebook chapter 8.09 and AOAC Official Method 993.12

APPENDIX 4: DISPOSAL

Invisible Sentinel devices are for single use only. Decontaminate all surfaces, media and reagents and discard in accordance with local, state, and federal regulations.