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
The control line, as indicated by the letter C on the Veriflow® LM Assay cassette, should always develop. The test line, as indicated by the letter T on the Veriflow® LM Assay 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 regulation.

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CUSTOMER SERVICE
Invisible Sentinel customer service and technical assistance can be reached Monday-Friday 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.

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

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.


SDS INFORMATION AVAILABLE
Safety Data Sheets (SDS) are available for this test kit and all of Invisible Sentinel's test kits by calling Invisible Sentinel at 215-966-6118.
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 (Sponges: 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) culture 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 culture 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 24-24.8 hours.

SECTION II: SWABS SURFACE SAMPLING AND ENRICHMENT (Sponges: see Section II)
1. If swabs are not pre-moistened, pipette 2 mL of Dey-Engley Neutralization broth to each swab placed in an 2 oz (58 mL) incubation bag.
2. Squeeze excess moisture from swab and remove from incubation bag.
3. Sample 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 24-24.8 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 24-24.8 hours.

SECTION IV: SAMPLING AND ENRICHMENT for 125 GRAM DELI MEAT SAMPLES
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 24-24.8 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 24-24.8 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°C ± 2°C, pre or post boil, for 1 week, prior to step 6 below.
5. Transfer 5 μl of liquid from cooled boiled sample from step 4 to Veriflow® LM PCR tube for each sample.
   a. Note: Open Veriflow® LM PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
6. Place Veriflow® LM PCR tube into IS PCR Thermocycler, select “VFLOWLM” program and press “START RUN”, as directed by the Thermocycler User Guide.
7. Upon completion of “VFLOWLM” program, press “STOP RUN”, and proceed to Cassette Sample Analysis step 1.

CASSETTE SAMPLE ANALYSIS
1. Remove tubes from IS PCR Thermocycler and add 4 drops of Buffer B directly to each Veriflow® LM PCR tube.
2. Transfer entire contents (200 μL) of Veriflow® LM PCR tube directly to Veriflow® LM Assay cassette sample window with pipette. A separate Veriflow® LM Assay cassette must be used for each Veriflow® LM PCR tube.
3. Allow Veriflow® LM Assay cassette to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of Buffer B directly to each Veriflow® LM Assay cassette sample window.
5. Allow Veriflow® LM Assay cassette to develop for 1 minutes ± 15 seconds.
   a. Note: Veriflow® LM Assay cassette may develop for up to 120 minutes before proceeding to step 6.
   a. The appearance of one red line (control) in the Veriflow® LM Assay cassette window indicates a negative result.
   b. The appearance of two red lines (control and test) in the Veriflow® LM Assay cassette window indicates a positive result.