THE POWER OF MOLECULAR DIAGNOSTICS IN THE PALM OF YOUR HAND®

APPENDIX 1: MATRICES TESTED
Surfaces: Stainless Steel, Sealed Concrete, Plastic, Ceramic Tile
Food (RTE): Deli Turkey Meat, Hot Dogs

APPASSAY PRINCIPLES
Veriflow® Listeria species (LS) is a molecular based assay for the presumptive and qualitative detection of Listeria species. 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® LS system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow® LS provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER
The Veriflow® LS system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with foodborne pathogen detection. Veriflow® LS is specifically designed to be easy-to-use and requires only basic training in microbiological and molecular biological sample handling.

APPENDIX 2: RESULTS INTERPRETATION
The control line, as indicated by the letter C on the Veriflow® LS cassette, should always develop. The test line, as indicated by the letter T on the Veriflow® LS cassette, will only develop in the event of a positive sample for Listeria species. 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 method for the detection of Listeria species from environmental samples. Enriched samples (un-boiled) from the Veriflow® procedure can be transferred to Fraser broth (100 μL to 10 mL volume) for confirmation following the necessary steps laid out in the USDA/FSIS Microbiology Laboratory Guidebook Chapter 8.09. Boiled samples from Sample Prep Section can also be assayed with Veriflow® LM IS Cat. No. IS1002 for the detection of Listeria monocytogenes, to test for the occurrence of this strain in a Listeria species positive sample.

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.

CUSTOMER SERVICE
Invisible Sentinel customer service and technical assistance can be reached Monday-Friday between 9AM and 5PM Eastern Standard 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
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MATERIALS PROVIDED
1. IS LS PCR Reagent - Cat. No. IS0505200
2. IS Buffer B - Cat. No. IS07002
3. IS Veriflow® Listeria Species Assay Cassette - Cat. No. IS0101
4. 1.5 mL Sample Boil Tubes - Cat. No. IS0935

MATERIALS PURCHASED SEPARATELY
- IS Listeria Broth – Cat. No. IS09302

MATERIALS NEEDED for ENVIRONMENTAL SAMPLE
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-Englyey broth.
2. Dey-Englyey broth (if necessary).
3. 18 oz. (532 mL) incubation bag for sponge samples.
4. 2 oz. (68 mL) incubation bag for swab samples.

MATERIALS NEEDED for READY TO EAT (RTE) SAMPLE
1. 24 oz. (710 mL) incubation bags (for Hot Dogs).
2. 55 oz. (1626 mL) incubation bags (for Deli Turkey Meat Samples).

OTHER MATERIALS NEEDED
1. Invisible Sentinel SimpliAmp PCR Thermocycler – Cat. No. ISTC002
2. Incubator that provides continuous and stable temperatures of 35°C ± 2°C
3. Water bath for boiling or heating block (95°C ± 2°C)
4. Heat tolerant beaker (optional for boiling)
5. Pipettes and tips for 5 μL, 200 μL and 1000 μL volumes
6. Glassware and autoclave for media prep
7. Racks for culture bags and 1.5 mL tubes
8. dH2O
9. Scale for weighing of sample and media

STORAGE of MATERIALS
The Veriflow® LS kit components, including cassettes, growth media and buffers should be stored at room temperature (20-25°C). The Veriflow® LS test PCR reagents should be stored at -20°C ± 2°C. Store autoclaved liquid media in the dark (photosensitive) at 4°C ± 2°C for a maximum of 30 days.

PRECAUTIONS
1. Enriched samples could contain Listeria monocytogenes, a human pathogen. All samples collected for use with the Veriflow® LS Assay should be handled with care.
2. Assay users should observe standard BSL-2 microbiological practices and safety precautions when performing this assay. Because high levels of enriched bacteria can result from use, immunocompromised individuals should avoid using the Veriflow® LS system.
3. Do not use Veriflow® LS Assay kit 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.
7. Do not retract cassette switch until steps 1 through 5 of the Cassette Sample Analysis section have been completed as directed.

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 ± 2°C in the dark for a maximum of 30 days).
2. Allow media to equilibrate to room temperature (20-25°C) before use.

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.

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. (532mL) incubation bag.
2. Squeeze excess moisture from sponge and remove from incubation bag.
3. Sample surface area of interest using sponge for 30 seconds.
4. Transfer sponge back to incubation bag.
5. Transfer 200 mL IS Listeria Broth into bag containing sponge used for sampling, seal, and briefly (5-10 seconds) massage sponge.
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 2.0 oz. (58mL) culture 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 briefly (5-10 seconds) massage swab.
6. Place bag into 35°C ± 2°C incubator, in rack, for 24-28 hours.

SECTION III: SAMPLING AND ENRICHMENT for 25 GRAM RTE SAMPLE
1. Weigh out 25 gram sample.
2. Transfer 25 gram sample to 24 oz. (710 mL) incubation bag.
3. Transfer 225 mL IS Listeria broth 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
2. Transfer 125 gram sample of Deli Meat to 55 oz. (1626 mL) incubation bag.
3. Transfer 375 mL IS Listeria broth 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.

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 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 the next step (step 5).
5. Transfer 5 μL of liquid from cooled sample from step 4 to Veriflow® LS PCR tube for each sample.
   a. Note: Open Veriflow® LS PCR tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
6. Place Veriflow® LS PCR tube into IS PCR Thermocycler, select “VFLOWLS” program and press “START RUN”, as directed by the Thermocycler User Guide.

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

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