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

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 26 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.

APPENDIX 1: MATRICES TESTED
25 GRAMS
Lava Cake
Cake Pops
Cookies
Chocolate Cake (non-layered)
Cheese Cake (pumpkin)
375 GRAMS
Lava Cake

APPENDIX 2: RESULTS INTERPRETATION
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 FDA/BAM method for the detection of Listeria monocytogenes from confectionery samples.

Enriched samples (un-boiled) from the Veriflow procedure can be transferred to secondary enrichment for confirmation following the necessary steps laid out in the FDA/BAM Chapter 10 method.

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.

STORAGE OF MATERIALS

The Veriflow® kit components, including cassettes, plastics, growth media and buffers should be stored at room temperature (20-25°C). The Veriflow® 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. Listeria monocytogenes is a human pathogen. All samples collected for use with the Veriflow® Assay should be handled with care.
2. Assay users should observe standard microbiological practices and practice good laboratory hygiene and safety precautions when performing this assay.
3. Do not use Veriflow® Assay cassettes past indicated expiration date.
4. Do not use enrichment broth past expiration date.
5. Use rehydrated enrichment broth within 30 days of preparation.
6. Deviations from the assay protocol may impact overall test performance.

NOTE: See Appendix 1 for matrices tested

MEDIA PREP

1. Add 36.1 grams modified Listeria enrichment broth media per 1 Liter dH₂O and autoclave for 15 minutes at 121°C. (Media is photosensitive and can be stored at 4°C for a maximum of 30 days).
2. Pre-warm media to 35°C ± 2°C before use.

SECTION I: SAMPLING AND ENRICHMENT for 25 GRAM SAMPLES

1. Weigh out 25 gram confectionery sample.
2. Transfer 25 gram sample to 24 oz. (710 ml) incubation bag.
3. Transfer 225 ml media prepared above to incubation bag from step 2.
4. Stomach 30 seconds to break down sample. This will allow penetration of the enrichment broth into the sample.
5. Place bag into 35°C ± 2°C incubator, in rack, for 26-28 hours.

SECTION II: SAMPLING AND ENRICHMENT for 375 GRAM LAVA CAKE SAMPLE

1. Weigh out 375 gram lava cake sample.
2. Transfer 375 gram sample to 55 oz. (1626 ml) incubation bag.
3. Transfer 1 liter 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 26-28 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°C ± 2°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 Veriflow® CONFECT program.

FLOWTHROUGH CASSETTE SAMPLE ANALYSIS

1. Place provided 1.5 ml PCR samples in tube rack (1 for each sample to be tested).
2. Transfer entire contents (200 µl) of PCR tube directly to Veriflow® Assay cassette sample window with pipette. A separate Veriflow® Assay cassette must be used for each PCR tube.
3. Allow test to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of BUFFER B directly to each Veriflow® Assay cassette sample window.
5. Allow test to develop for 1 minute ± 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.
7. The appearance of one red line (control and test) in the cassette window indicates a negative result.
8. The appearance of two red lines (control and test) in the cassette window indicates a positive result.
9. 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 Invisible Sentinel's Food test kits by calling Invisible Sentinel at 215-966-6118.

NOTE: See Appendix 1 for matrices tested