APPENDIX 1: ASSAY APPLICABILITY

Meat, Produce and Dairy:
- 20% Fat Ground Beef
- 2% Milk
- Spinach

APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the Veriflow® O157:H7 test cassette, should always develop. The test line, as indicated by the letter T on the Veriflow® O157:H7 cassette, will only develop in the event of a positive sample for E. coli O157:H7. If the control line fails to develop, the test is invalid, and will need to be repeated.

APPENDIX 3: CONFIRMATION OF RESULT

Presumptive positive samples must be confirmed by the FDA BAM Chapter 4A or the USDA/FSIS MLG chapter 5.08 methods for the detection of E. coli O157:H7 in dairy, meat and produce, following an immuno-magnetic separation (IMS) procedure.

Note: Use up-to-date methods for confirmations. Enriched samples (un-boiled) from the Veriflow® procedure can be transferred to selective enrichment for confirmation following the necessary steps laid out in the FDA BAM or USDA/FSIS methods.

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.

V. ISO9453

ASSAY PRINCIPLES

Veriflow® O157:H7 is a molecular based assay for the presumptive and qualitative detection of Eschericia coli O157:H7. 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 18 hours of incubation for maximum sensitivity.

The Veriflow® O157:H7 system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow® O157:H7 provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER

The Veriflow® O157:H7 system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with foodborne pathogen detection. Veriflow® O157:H7 is specifically designed to be easy-to-use and eliminates the need for advanced training in molecular biology.

**MATERIALS PROVIDED**
1. IS O157:H7 PCR Tube - Cat. No. IS0509200
2. IS Buffer MP - Cat. No. IS0713
3. IS Veriflow® O157:H7 Assay Cassette - Cat. No. IS0101
4. IS 1.5 mL Sampling Tubes - Cat. No. IS0320

**MATERIALS PURCHASED SEPARATELY**
- Invisible Sentinel SimpliAmp PCR Thermocycler – Cat. No. ISTC002
- IS mTSB Broth - Cat. No. IS0322
- IS mBPWp Broth - Cat. No. IS0320

**MATERIALS NEEDED**
- LoveToGrow®
- e. info@invisiblesentinel.com | www.invisiblesentinel.com
- f. 215.386.3970 | p. 215.966.6118

**PRECAUTIONS**
- PCR tubes should be stored at -20°C ± 2°C.

**CAUTIONS**
- Do not use Veriflow® with care.
- All samples are human pathogens. 
- Escherichia coli O157:H7 assay should be handled with care.

**NOTE: See Appendix 1 for matrices tested**

- For meat samples, go to sections I.
- For dairy samples, go to section II.
- For leafy produce samples, go to section III.

**SECTION I**
**SAMPLING and ENRICHMENT for 325 and 375 GRAM MEAT SAMPLES**
1. Transfer sample to 92 oz. (2721 mL) incubation bag.
2. Transfer entire contents (200 g) to IS 1.5 mL Sampling Tubes - Cat. No. IS0935
3. Media can be stored at room temperature (20-25°C) for 30 days.
4. Note: Pre-warm media to 42°C ± 2°C before use.

**SECTION II**
**SAMPLING and ENRICHMENT for DAIRY SAMPLES**
1. Transfer 25 gram or 25 mL sample to 24 oz. (710 ml) incubation bag.
2. Add 300 mL pre-warmed mBPWp broth to 24 oz. incubation bag containing sample.
3. Stomach incubation bag containing sample for 1-2 minutes to break down sample.
4. Transfer incubation bag to an incubator set to 42°C ± 2°C, in rack, for 18-24 hours.

**SECTION III**
**SAMPLING and ENRICHMENT for LEAFY PRODUCE**
1. Transfer 200 gram leafy produce to 92 oz. (2721 mL) incubation bag.
2. Add 300 mL of enriched culture from step 2 into prepared 1.5 mL PCR tube for each sample (thaw PCR tube for 10 ± 1 minutes at room temperature [20-25°C] and use immediately).

**CASSETTE SAMPLE ANALYSIS**
1. Remove tubes from IS PCR Thermocycler and add 4 drops of Buffer MP to each IS O157:H7 PCR tube.
2. Note: Invert Buffer MP bottle before each use to distribute any settled contents.
3. Transfer entire contents (200 µL) of Veriflow® O157:H7 PCR tube directly to each Veriflow® O157:H7 cassette sample window.
4. A separate Veriflow® O157:H7 cassette must be used for each Veriflow® O157:H7 PCR tube.
5. Allow Veriflow® O157:H7 cassette to develop for 2 minutes ± 15 seconds.
6. Add 4 drops of Buffer MP directly to each Veriflow® O157:H7 cassette sample window.
7. Allow Veriflow® O157:H7 cassette to develop for 1 minute ± 15 seconds.
8. Optional: Veriflow® O157:H7 cassette can be developed for up to 120 minutes before proceeding to step 6.
10. The appearance of one red line (control) in the Veriflow® O157:H7 cassette sample window indicates a negative result.
11. The appearance of two red lines (control and test) in the Veriflow® O157:H7 cassette sample window indicates a positive result.