



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

ASSAY PRINCIPLES

Veriflow[®] Escherichia coli O157:H7 is a molecular based assay for the presumptive and qualitative detection of *E. coli* O157:H7. The assay utilizes a multiplex 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.

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MATERIALS PROVIDED

1. IS O157:H7 PCR Reagent - Cat. No. IS0509
2. IS BUFFER MP- Cat. No. IS0713
3. IS Veriflow[®] Assay Cassette - Cat. No. IS0101EC
4. 1.5 ml Sampling Tubes - Cat. No. IS0943
5. IS mTSB Broth - Cat. No. IS0310
6. IS mBPWp Broth - Cat. No. IS0311

MATERIALS PURCHASED SEPARATELY

MATERIALS NEEDED

1. 24 oz. (710 ml) incubation bags (for Leafy Produce and Milk Samples)
2. 92 oz. (2721 ml) incubation bags (for Ground Beef and Beef Trim Samples)
3. Butterfield's phosphate buffer (for produce rinses)
4. Incubator that provides continuous and stable temperatures of $42^{\circ}\text{C} \pm 2^{\circ}\text{C}$
5. Water bath for boiling or heating block ($95^{\circ}\text{C} \pm 2^{\circ}\text{C}$)
6. Heat tolerant beaker
7. PCR Thermocycler
8. Pipettes and tips for 5, 200, and 250 μl volumes
9. Glassware and autoclave for media prep
10. Racks for culture bags and 1.5 ml tubes
11. dH_2O
12. Scale for weighing of sample and media

STORAGE OF MATERIALS

The Veriflow[®] O157:H7 kit components, including cassettes, plastics, growth media and buffers should be stored at room temperature ($20\text{-}25^{\circ}\text{C}$). The Veriflow[®] O157:H7 test PCR reagents should be stored at $-20^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

PRECAUTIONS

1. *Escherichia coli* O157:H7 bacteria are human pathogens. All samples collected for use with the Veriflow[®] O157:H7 assay should be handled with care.
2. Assay users should observe standard BSL-2 microbiological practices and safety precautions when performing this assay. Because of high levels of enriched bacteria can result from use, immunocompromised individuals should avoid using the Veriflow[®] O157:H7 system.
3. Do not use Veriflow[®] O157:H7 assay cassettes past indicated expiration date.
4. Do not use media past indicated expiration date.
5. Use rehydrated media within 30 days of preparation.
6. Deviations from the assay protocol may impact overall test performance.

MEDIA PREP

mTSB – For beef trim and ground beef matrices

1. Add 43 grams mTSB broth per 1 Liter dH₂O
2. Autoclave at 121°C for 15 minutes.
3. Media can be stored at room temperature for 30 days.
4. Note: **Pre-warm** media to 42°C ± 2°C before use.

mBPWp – For milk and spinach matrix

1. Add 42 grams mBPWp broth per 1 Liter dH₂O.
2. Autoclave at 121°C for 15 minutes.
3. Media can be stored at room temperature for 30 days.
4. Note: **Pre-warm** media to 42°C ± 2°C before use.

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 GRAM MEAT SAMPLES

1. Weigh out 325 gram sample.
2. Transfer 325 gram sample to 92 oz. (2721 ml) incubation bag.
3. Transfer 1625 ml pre-warmed mTSB to 92 oz. incubation bag containing sample.
4. Stomach to break down sample.
5. Place bag into 42°C ± 2°C incubator, in rack, for 18-24 hours.

SECTION II:

SAMPLING and ENRICHMENT for DAIRY SAMPLES

1. Transfer 25 gram or 25 mL sample to 24 oz. (710 ml) incubation bag.
2. Transfer 225 ml pre-warmed mBPWp to 24 oz. incubation bag containing sample.
3. Stomach to break down sample.
4. Place bag into 42°C ± 2°C incubator, in rack, for 18-24 hours.

SECTION III:

SAMPLING and ENRICHMENT for LEAFY PRODUCE RINSES

1. Combine 200 grams leafy produce with 450 mL mBPWp in 92 oz. (2721 mL) incubation bag.
2. Agitate bag briefly to rinse produce, do not pummel or stomach sample.
3. Place bag into 42°C ± 2°C incubator, in rack, for 18-24 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 1.5 ml sampling tube and seal.
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. Transfer sample PCR tube to thermocycler and run VFLOWEC Program.

FLOWTHROUGH CASSETTE SAMPLE ANALYSIS

1. Remove tubes from thermocycler.
2. **Invert BUFFER MP bottle** before each use to distribute any settled contents, and add 4 drops of BUFFER MP directly to each PCR tube.
3. Transfer entire contents (200 µl) of PCR tube directly to Veriflow[®] **O157:H7** assay cassette sample window with pipette. A separate Veriflow[®] **O157:H7** assay cassette must be used for each PCR tube.
4. Allow test to develop for 2 minutes \pm 15 seconds.
5. Add 4 drops of BUFFER MP directly to each Veriflow[®] **O157:H7** assay cassette sample window.
6. Allow test to develop for 1 minute \pm 15 seconds.
 - a. Optional: test may develop for up to 120 minutes before retraction and recording of results.
7. 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.



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APPENDIX 1: ASSAY APPLICABILITY

Meat, Produce and Dairy:

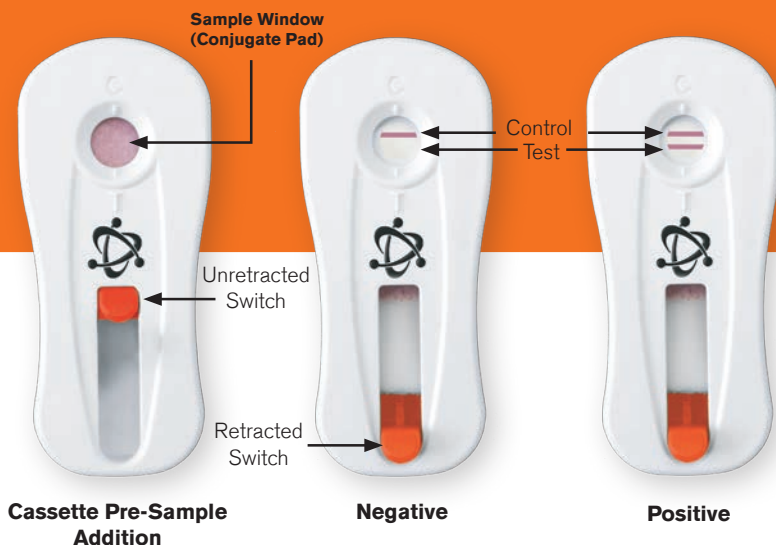
20% Fat Ground Beef

Beef Trim

Milk

Spinach

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 *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 RESULTS

Presumptive positive samples must be confirmed by the FDA BAM method for the detection of *E. coli* O157:H7 in dairy, meat and produce.

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 Chapter 4a procedure.

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 between 9:00 AM and 5:00 PM EST 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.