



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



### ASSAY PRINCIPLES

Veriflow® *Campylobacter* is a molecular based assay for the presumptive and qualitative detection of the most common occurring foodborne *Campylobacter* species: *Campylobacter jejuni* and *Campylobacter coli*. 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 non-specialized incubation for maximum sensitivity. The Veriflow® *Campylobacter* system eliminates the need for microaerobic chambers, gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow® *Campylobacter* provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

### INTENDED USER

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

*Invisible Sentinel® and Veriflow® are trademarks of Invisible Sentinel, Inc., of Philadelphia, PA. Stomache® is a registered trademark of Tekmar, Inc., Cincinnati, OH. U.S. Patent No. 8,183,059 and other patents pending. Purchase and use of this product is subject to Invisible Sentinel's Terms and Conditions of Sale located at <http://www.invisiblesentinel.com>.*



Phone 215.966.6118 | Fax 215.386.3970

Email [info@invisiblesentinel.com](mailto:info@invisiblesentinel.com) | [www.invisiblesentinel.com](http://www.invisiblesentinel.com)

## MATERIALS PROVIDED

1. IS *Campylobacter* PCR Reagent - Cat. No. IS0501
2. IS BUFFER B - Cat. No. IS0702
3. IS Veriflow<sup>®</sup> *Campylobacter* Assay Cassette - Cat. No. IS0101
4. 1.5 ml Sample Boil Tubes - Cat. No. IS0902
5. IS Enhanced Bolton Broth - Cat. No. IS0301
6. IS BPW Broth - Cat. No. IS0305
7. 50 ml Conical Tubes - Cat. No. IS0901

## MATERIALS NEEDED, PER CHICKEN CARCASS

1. Selective supplement (e.g. EMD #1.00079.0017 or equivalent)
  - a. Supplements dissolved in 50% ethanol
2. 2 gallon Stomacher<sup>®</sup> or equivalent bag
3. Incubator that provides continuous and stable temperatures of 37°C ± 1°C and 42°C ± 1°C
4. Hot plate/heat source for boiling water bath
5. Heat tolerant beaker
6. PCR Thermocycler
7. Pipettes and tips for 5, 200 and 1000 µl volumes
8. 50 ml or 25 ml Serological pipettes
9. Glassware and autoclave for media prep
10. 70% isopropanol (IPA) spray bottle (optional)
11. Racks for 50 ml conical and 1.5 ml tubes
12. dH<sub>2</sub>O

## STORAGE OF MATERIALS

The Veriflow<sup>®</sup> *Campylobacter* kit components, including cassettes, plastics, growth media and buffers should be stored at room temperature (20-25°C). The IS *Campylobacter* PCR Reagent should be stored at -20°C ± 1°C.

## PRECAUTIONS

1. *Campylobacter jejuni* and *Campylobacter coli* are human pathogens. All samples collected for use with the Veriflow<sup>®</sup> *Campylobacter* Assay should be handled with care.
2. Assay users should observe standard microbiological practices and safety precautions when performing this assay.
3. Do not use Veriflow<sup>®</sup> *Campylobacter* Assay cassettes past indicated expiration date.
4. Do not use IS Enhanced Bolton Broth past indicated expiration date.
5. Use rehydrated IS Enhanced Bolton Broth within 24 hours of preparation.
6. Ensure 50 ml conical tubes are tightly capped to ensure optimal growth conditions.
7. Deviations from the assay protocol may impact overall test performance.

## MEDIA PREPARATION AND CARCASS RINSE

1. Media Prep A: Add 58.0 grams IS Enhanced Bolton Broth per 1 Liter dH<sub>2</sub>O (2X concentration).
2. Media Prep B: Add 20.0 grams IS BPW broth per 1 Liter dH<sub>2</sub>O.
3. Autoclave rehydrated media from steps 1 and 2 above for 15 minutes and allow to cool to room temperature (20-25°C). (Use IS Enhanced Bolton broth media within 24 hours of preparation).
4. Transfer 27 ml 2X IS Enhanced Bolton broth media from Step 1 to a 50 ml conical tube.
5. Prepare the selective supplement according to the manufacturer's instructions. Determine the volume of selective supplement required for a 54 ml final culture volume. Add this volume of selective supplement to the 27 ml of rehydrated IS Enhanced Bolton Broth contained in the 50 ml conical tube (e.g. 540 µl for EMD #1.00079.0017).
6. Place chicken carcass in 2 gallon Stomacher<sup>®</sup> or equivalent bag.
  - a. If chicken is packaged, generously spray chicken package down with 70% IPA before opening.

7. Pour 400 ml rehydrated IS BPW broth from Step 3 into bag with carcass. Seal bag and agitate sample for 1 to 2 minutes.
8. Pipette 27 ml of chicken carcass rinse from Step 7 into the 50 ml conical tube from Step 5 for a final 54 ml volume. Tightly cap conical tube and invert twice to mix sample.

## INCUBATION (for maximum sensitivity)

1. Place 50 ml conical tube from MEDIA PREPARATION AND CARCASS RINSE Step 8 into an incubator set to  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 4 hours.
2. After 4 hours at  $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ , transfer 50 ml conical tube to an incubator set to  $42 \pm 1^{\circ}\text{C}$  for an additional 20 hours.

## SAMPLE PREP and PCR

1. Place provided 1.5 ml tubes in rack (1 for each sample to be tested).
2. Remove culture conical tube from incubator and invert twice to re-suspend settled tube contents.
3. Remove cap of culture conical tube and pipette 500  $\mu\text{l}$  of enriched culture from step 2 into prepared 1.5 ml tube from Step 1.
4. Boil sample from Step 3 in water bath for 10 minutes and allow to cool to room temperature ( $20\text{-}25^{\circ}\text{C}$ ).
  - a. NOTE: Samples can be stored sealed at  $-20^{\circ}\text{C}$ , pre or post boil, for 1 week, prior to Step 5.
5. Transfer 5  $\mu\text{l}$  of liquid from cooled boiled sample from Step 4 to thawed PCR reagent tube for each sample.
  - a. Open PCR tube only when adding sample and promptly close, to avoid cross contamination between tubes.
6. Cycle sample PCR tube in thermocycler with VFLOWCA program.

## FLOWTHROUGH CASSETTE SAMPLE ANALYSIS

1. Remove tubes from thermocycler and add 4 drops of **BUFFER B** directly to each PCR tube.
2. Transfer entire contents (200  $\mu\text{l}$ ) of PCR tube directly to Veriflow<sup>®</sup> *Campylobacter* Assay cassette sample window with pipette. A separate Veriflow<sup>®</sup> *Campylobacter* Assay cassette must be used for each PCR tube.
3. Allow test to develop for 2 minutes  $\pm$  15 seconds.
4. Add 4 drops of **BUFFER B** directly to each Veriflow<sup>®</sup> *Campylobacter* Assay cassette sample window.
5. 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.
6. Retract switch and record results.
  - a. The appearance of one red line (control) in the test window indicates a negative result.
  - b. The appearance of any two red lines (control and test) in the test window indicates a positive result.

## DISPOSAL

Invisible Sentinel devices are for single use only. Dispose of test devices, tubes, and media in accordance with all applicable local, state, and federal regulations.

## 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 a Invisible Sentinel sales or technical representative. Training on this product and all Invisible Sentinel test kits is available.

## MSDS INFORMATION AVAILABLE

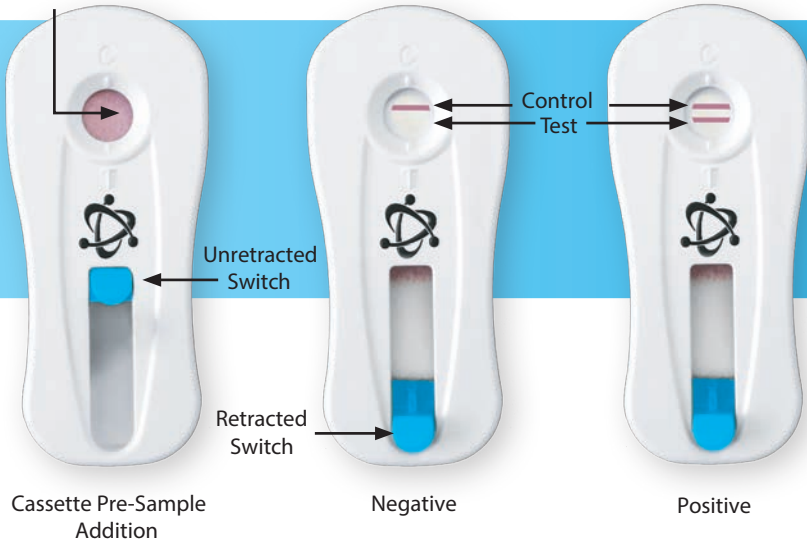
Material Safety Data Sheets (MSDS) are available for this test kit and all of Invisible Sentinel's Food test kits by calling Invisible Sentinel at 215-966-6118.

## APPENDIX 1: ASSAY APPLICABILITY

Veriflow® *Campylobacter* has been approved for the detection of *C. jejuni* and *C. coli* in chicken carcass rinsates.

## APPENDIX 2: RESULTS INTERPRETATION

Sample Window  
(Conjugate Pad)



The control line, as indicated by the letter C on the Veriflow® *Campylobacter* Assay cassette, should always develop. The test line, as indicated by the letter T on the Veriflow® *Campylobacter* Assay cassette, will only develop in the event of a positive sample for *Campylobacter jejuni* or *Campylobacter coli*. 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 current USDA/FSIS method for the detection of *Campylobacter* from poultry rinses.

Samples (un-boiled) from the chicken carcass rinse achieved using this protocol can be used for the confirmation, following the necessary steps laid out in the USDA/FSIS Microbiology Laboratory Guidebook chapter 41.01 (as may be updated, amended or superseded from time to time).

## APPENDIX 4: DISPOSAL

Decontaminate all media and reagents and discard in accordance with applicable local, state, and federal regulations.