

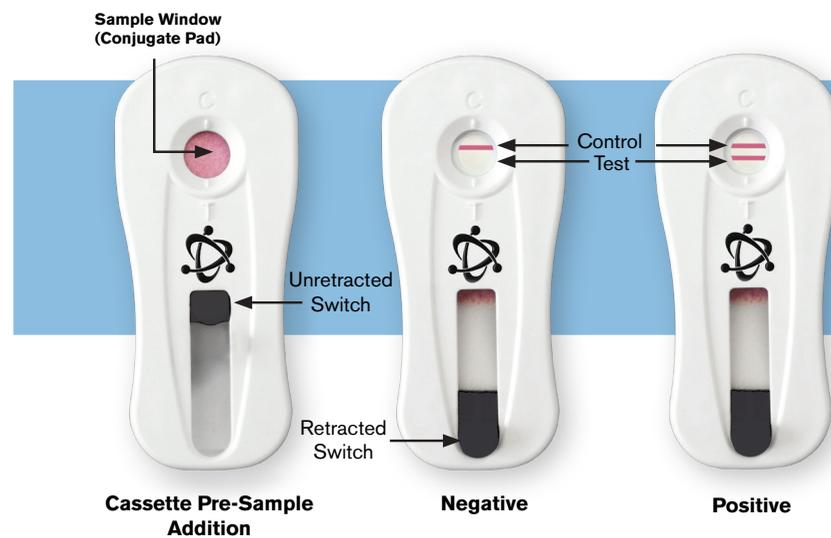
6. Transfer 5 μ L from *Legionella* PCR Reagent A tube to *Legionella* PCR Reagent B tube.
 - a. Note: Open *Legionella* PCR Reagent B tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
7. Place *Legionella* PCR Reagent B tube into Invisible Sentinel PCR Thermocycler, select "*Legionella* PCR B" program and press "START RUN", as directed by the Thermocycler User guide.
8. Upon completion of "*Legionella* PCR B" program, press "STOP RUN"; remove *Legionella* PCR Reagent B tube from Invisible Sentinel PCR Thermocycler and proceed to Cassette Sample Analysis.

CASSETTE SAMPLE ANALYSIS

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

APPENDIX 1: RESULTS INTERPRETATION

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



APPENDIX 2: 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. IS2068.0



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THE POWER OF
MOLECULAR DIAGNOSTICS
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ASSAY PRINCIPLES

Veriflow® *Legionella* is a molecular based assay for the presumptive and qualitative detection of *Legionella* species in water and environmental swab samples without enrichment. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes post PCR amplification. The Veriflow® *Legionella* system eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, and does not require complex data analysis. Veriflow® *Legionella* provides the specificity and sensitivity of PCR based amplification in a cost-efficient and easy-to-use format.

INTENDED USER

The Veriflow® *Legionella* system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with human pathogen detection. Veriflow® *Legionella* is specifically designed to be easy-to-use and require only basic training in microbiological and molecular biological sample handling.

Invisible Sentinel® and Veriflow® are trademarks of Invisible Sentinel, Inc, of Philadelphia, PA, 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>.



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MATERIALS PROVIDED

1. IS *Legionella* PCR Reagent A - Cat. No. IS0535
2. IS *Legionella* PCR Reagent B – Cat No. IS0539
3. IS Buffer A- Cat No. IS0701
4. IS Buffer B- Cat. No. IS0702
5. IS Veriflow® *Legionella* Assay Cassettes - Cat. No. IS0101
6. Black microcentrifuge tubes - Cat No. IS0995
7. Clear microcentrifuge tubes - Cat No. IS0996

MATERIALS NEEDED

1. Invisible Sentinel SimpliAmp PCR Thermocycler – Cat No. ISTC002
2. LED Photolysis Device – Cat No. ISCT003
3. Pipettes and tips capable of transferring between 2 µL and 1000 µL volumes
4. Pipettes capable of transferring between 4.5mL and 25 mL volumes
5. Ethidium Monoazide
6. Centrifuge compatible with 50 mL conical tubes and capable of 3000 x g
7. 50 mL conical tubes (capable of withstanding 3000 x g centrifuge speed)
8. Environmental swabs (for environmental testing)
9. End-over-end mixer for 1.5 mL tubes
10. dH₂O
11. Vortex
12. 100% Ethanol

STORAGE OF MATERIALS

The Veriflow® *Legionella* kit components, including cassettes and buffers should be stored at room temperature (20–25°C). The Veriflow® *Legionella* test PCR Reagents A and B should be stored at -20°C ± 2°C.

PRECAUTIONS

1. Certain *Legionella* strains are human pathogens. All samples collected for use with the Veriflow® *Legionella* assay should be handled with care. Assay users should observe standard BSL-2 microbiological practices and safety precautions when performing this assay. Immunocompromised individuals should avoid using the Veriflow® *Legionella* system.
2. Do not use Veriflow® *Legionella* assay kit past indicated expiration date.
3. Deviations from the assay protocol may impact overall test performance.
4. Do not retract cassette switch until Steps 1 through 5 of the Cassette Sample Analysis section have been completed as directed.

DIFFERENTIATION SOLUTION PREP (OPTIONAL)

1. Add 595 µL of 100% ethanol to 5 mg of lyophilized ethidium monoazide to dissolve.
2. Add 25 µL of ethidium monoazide solution to 75 µL of dH₂O in a provided black tube.
3. Label tube as Differentiation Solution.
4. Store at 4° C when not in use (when protected from light, preparation stable for 6 months).

WATER SAMPLE PREP and PCR

1. Transfer 25 mL of water sample to a 50 mL conical tube.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 3000 x g.
3. Remove 24.5 mL supernatant from 50 mL tube (careful not to disturb pellet).
4. Resuspend pellet in 50 mL conical tube with the remaining 500 µL of sample by mixing until the pellet is no longer visible.
 - a. Optional: Viability Differentiation
 - b. Transfer 500 µL of resuspension to provided black microcentrifuge tube, and add 2.5 µL of Differentiation Solution.
 - c. Incubate treated sample for 5 minutes on an end-over-end mixer, at room temperature.
 - d. Transfer sample to provided transparent microcentrifuge tube and incubate sample for 15 minutes in LED Photolysis Device.
 - e. Add 500 µL of Buffer A to sample and centrifuge at 3000 x g for 10 minutes.
 - f. Remove 950 µL of supernatant with pipette, being careful not to disturb pellet.
 - g. Resuspend in 100 µL of Buffer A.
5. Transfer 5 µL from resuspended pellet generated in Step 4 to *Legionella* PCR Reagent A tube.
 - a. Note: Open *Legionella* PCR Reagent A tube only when adding sample and promptly close after to avoid cross contamination between tubes.
6. Place *Legionella* PCR Reagent A tube into Invisible Sentinel PCR Thermocycler, select “*Legionella* PCR A” program and press “START RUN” as directed by the Thermocycler user guide.
7. Upon completion of “*Legionella* PCR A” program, press “STOP RUN” and remove *Legionella* PCR Reagent A tube from Invisible Sentinel PCR Thermocycler.
8. Transfer 5 µL from *Legionella* PCR Reagent A tube to *Legionella* PCR Reagent B tube.
 - a. Note: Open *Legionella* PCR Reagent B tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
9. Place *Legionella* PCR Reagent B tube into Invisible Sentinel PCR Thermocycler, select “*Legionella* PCR B” program and press “START RUN”, as directed by the Thermocycler User guide.
10. Upon completion of “*Legionella* PCR B” program, press “STOP RUN”, remove *Legionella* PCR Reagent B tube from Invisible Sentinel PCR Thermocycler and proceed to Cassette Sample Analysis.

ENVIRONMENTAL SWAB SAMPLE PREP AND PCR

1. Add processed swab to 50 mL conical tube with 5 mL of water.
 - a. Water can be sterile or from the area of interest.
2. Vortex tube for 15 seconds and remove swab.
3. Centrifuge 50 mL conical tube with sample for 10 minutes at 3000 x g.
4. Remove 4.5 mL supernatant from 50 mL conical tube (careful not to disturb pellet).

5. Resuspend pellet in 50 mL tube with the remaining 500 µL of sample supernatant by mixing until the pellet is no longer visible.
 - a. Optional: Viability Differentiation
 - b. Transfer 500 µL of resuspension to black microcentrifuge tube and add 2.5 µL of Differentiation Solution.
 - c. Incubate treated sample for 5 minutes on an end-over-end mixer, at room temperature.
 - d. Transfer sample to provided transparent microcentrifuge tube and incubate sample for 15 minutes in LED Photolysis Device.
 - e. Add 500 µL of Buffer A and centrifuge sample at 3000 x g for 10 minutes.
 - f. Remove 950 µL of supernatant with pipette.
 - g. Resuspend in 100 µL of Buffer A.
6. Transfer 5 µL from resuspended pellet in Step 5 to *Legionella* PCR Reagent A tube.
7. Place *Legionella* PCR Reagent A tube into Invisible Sentinel PCR Thermocycler, select “*Legionella* PCR A” program and press “START RUN” as directed by the Thermocycler user guide.
8. Upon completion of “*Legionella* PCR A” program, press “STOP RUN” and remove *Legionella* PCR Reagent A tube from Invisible Sentinel PCR Thermocycler.
9. Transfer 5 µL from *Legionella* PCR Reagent A tube to *Legionella* PCR Reagent B tube.
 - a. Note: Open *Legionella* PCR Reagent B tube only when adding sample and promptly close after, to avoid cross contamination between tubes.
10. Place *Legionella* PCR Reagent B tube into Invisible Sentinel PCR Thermocycler, select “*Legionella* PCR B” program and press “START RUN”, as directed by the Thermocycler User guide.
11. Upon completion of “*Legionella* PCR B” program, press “STOP RUN”, remove *Legionella* PCR Reagent B tube from Invisible Sentinel PCR Thermocycler and proceed to Cassette Sample Analysis.

COLONY SAMPLE PREP and PCR

1. Pick and transfer desired colony into 500 µL of dH₂O in a 1.5 mL microcentrifuge tube.
2. Mix contents by pipetting up and down or by vortexing.
3. Transfer 5 µL to *Legionella* PCR Reagent A tube.
 - a. Note: Open *Legionella* PCR Reagent A tube only when adding sample and promptly close after to avoid cross contamination between tubes.
4. Place *Legionella* PCR Reagent A tube into Invisible Sentinel PCR Thermocycler, select “*Legionella* PCR A” program and press “START RUN” as directed by the SimpliAmp Thermocycler user guide.
5. Upon completion of “*Legionella* PCR A” program, press “STOP RUN” and remove *Legionella* PCR Reagent A tube from Invisible Sentinel PCR Thermocycler.