

APPENDIX 1: ASSAY APPLICABILITY

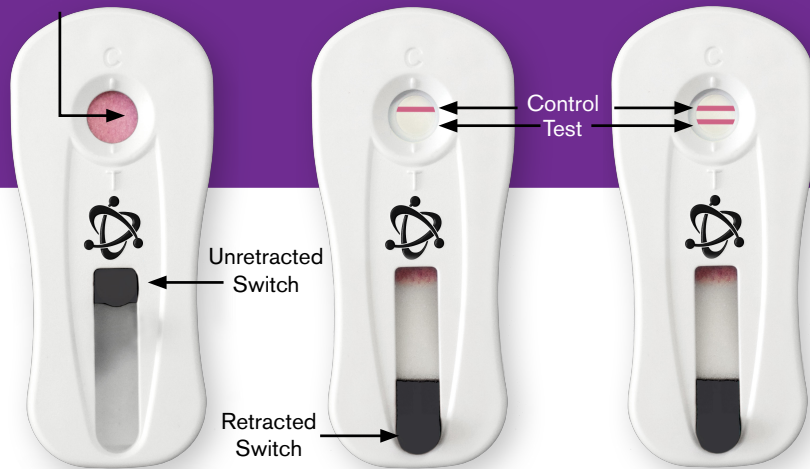
The Veriflow® ACB assay has been validated for the detection of Alicyclobacillus species in juice and related beverage products.

APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the Veriflow® ACB Assay cassette, should



Sample Window
(Conjugate Pad)



Cassette Pre-Sample
Addition

Negative

Positive

always develop. The test line, as indicated by the letter T on the Veriflow® ACB Assay cassette, will only develop in the event of a positive sample for Alicyclobacillus species. 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 can be confirmed by plating and colony PCR.

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.



V.I.S0969.3

THE POWER OF MOLECULAR DIAGNOSTICS IN THE PALM OF YOUR HAND

ASSAY PRINCIPLES

Veriflow® Alicyclobacillus species (ACB) is a molecular based assay for the qualitative and quantitative detection of Alicyclobacillus species. The assay utilizes a PCR detection method coupled with a rapid, visual, flow-based assay that develops in 3 minutes post PCR amplification, and generates results without enrichment or DNA purification. Veriflow® ACB eliminates the need for gel electrophoresis or fluorophore based detection of target amplifications, does not require complex data analysis, and allows for direct sampling to final analysis within 3 hours. Veriflow® ACB provides the specificity and sensitivity of PCR based amplification in a cost- efficient and easy-to-use format.

INTENDED USER

The Veriflow® ACB system is intended for use by personnel familiar with basic sample collection and preparation techniques associated with spoilage organism detection. Veriflow® ACB 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. 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 www.invisiblesentinel.com.



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MATERIALS PROVIDED

1. IS Veriflow® ACB PCR Tube – Cat. No. IS0521200
2. IS Buffer ACB – Cat. No. IS0714
3. IS Buffer B – Cat. No. IS0702
4. IS Veriflow ACB Assay Cassette – Cat. No. IS0101
5. 1.5 mL Sample Tube – Cat. No. IS0935

OTHER MATERIALS NEEDED

1. Invisible Sentinel SimpliAmp PCR Thermocycler – ISTC002
2. Centrifuge compatible with 50 mL conical tubes, capable of 3000 x g
3. Pipettes and Tips capable of 5 µL, 50 µL, 200 µL, and 25 mL volume transfers.

STORAGE OF MATERIALS

The Veriflow® ACB kit components, including cassettes and buffers should be stored at room temperature (20-25°C). The Veriflow® ACB PCR Tubes should be stored at -20°C ± 2°C

PRECAUTIONS

1. Assay users should observe standard microbiological practices and safety precautions when performing this assay.
2. Do not use Veriflow® ACB 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.

SAMPLE PREPARATION and PCR

1. Transfer 5 mL of sample to a 50 mL conical tube containing 20 mL of dH₂O. Mix thoroughly by inverting until homogenous.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 3000 x g.
3. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
4. Resuspend pellet in 50 mL conical tube with 1 mL of Buffer ACB using a vortexer for 5-10 seconds until the pellet is no longer visible.
5. Transfer 50 µL from resuspended pellet in 50 mL conical tube generated in step 4 to provided 1.5 mL sample tube and vortex 5-10 seconds.
6. Transfer 5 µL from 1.5 mL sample tube in step 5 to Veriflow® ACB PCR Tube.
 - a. Open Veriflow® ACB PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
7. Place Veriflow® ACB PCR Tube into IS PCR Thermocycler, select “VFLOWACB” program and press “START RUN”, as directed by the Thermocycler User Guide.
8. Upon completion of “VFLOWACB” program, press “STOP RUN”, and proceed to Cassette Sample Analysis step 1.

COLONY SAMPLE PREP and PCR

1. Pick and transfer colony into provided 1.5 mL sample tube.
2. Mix contents by pipetting sample up and down or by vortexing.
3. Transfer 5 µL of colony re-suspension to Veriflow® ACB PCR Tube.
 - a. Open Veriflow® ACB PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
4. Place Veriflow® ACB PCR Tube into IS PCR Thermocycler, select “VFLOWACB” program and press “START RUN”, as directed by the Thermocycler User Guide.
5. Upon completion of “VFLOWACB” program, press “STOP RUN”, and proceed to Cassette Sample Analysis step 1.

CASSETTE SAMPLE ANALYSIS

1. Remove tubes from IS PCR Thermocycler and add 4 drops of Buffer B directly to each Veriflow® ACB PCR Tube.
2. Transfer entire contents (200 µL) of Veriflow® ACB PCR Tube directly to Veriflow® ACB Assay cassette sample window with pipette. A separate Veriflow® ACB Assay cassette must be used for each Veriflow® ACB PCR Tube.
3. Allow Veriflow® ACB Assay cassette to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of Buffer B directly to each Veriflow® ACB Assay cassette sample window.
5. Allow Veriflow® ACB Assay cassette to develop for 1 minute ± 15 seconds.
 - a. Note: Veriflow® ACB Assay cassette can be developed for up to 120 minutes before proceeding to step 6.
6. Retract Veriflow® ACB Assay cassette switch and record results.
 - a. The appearance of one red line (control) in the Veriflow® ACB Assay cassette sample window indicates a negative result.
 - b. The appearance of two red lines (control and test) in the Veriflow® ACB Assay cassette sample window indicates a positive result.
 - i. For quantitative results scan cassette with IS Optical Reader.

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.