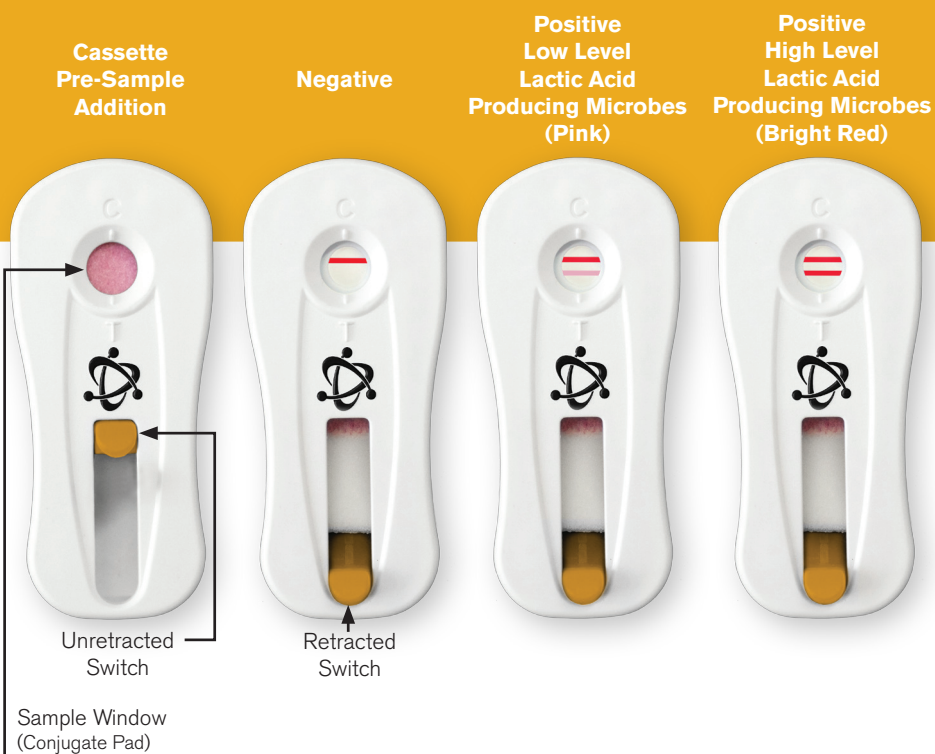


APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the brewLAP™ cassette, should always develop. The test line, as indicated by the letter T on the brewLAP™ cassette, will only develop in the event of a positive sample for *lactic acid-producing microbes*. If the control line fails to develop, the test is invalid, and will need to be repeated.



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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.

ASSAY PRINCIPLES

brewLAP™ is a molecular based assay for the qualitative detection of *lactic acid-producing microbes capable* of causing spoilage in beer. 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. brewLAP™ eliminates the need for gel electrophoresis or fluorophore-based detection of target amplification and provides same day results in under 3 hours. Ultimately, brewLAP™ provides the specificity and sensitivity of PCR based amplification in a cost-effective and easy-to-use format.

INTENDED USER

brewLAP™ is intended for use by personnel familiar with basic sample collection and preparation techniques associated with spoilage organism detection during fermentation and bottling. brewLAP™ 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, 8,476,082 and 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>.



V. IS0989.1



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MATERIALS PROVIDED

1. IS brewLAP™/vinoPAL™ PCR Tube – Cat. No. IS0533200
2. IS Buffer A – Cat. No. IS0701
3. IS Buffer B – Cat. No. IS0702
4. IS brewLAP™ Assay Cassette – Cat. No. IS0121

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, and 250 µL volume transfers
4. 50 mL conical tubes (capable of being centrifuged at 3000 x g)

STORAGE OF MATERIALS

The brewLAP™ kit components, including cassettes and buffers (Buffer A and B) should be stored at room temperature (20-25°C). The Veriflow® brewLAP™/vinoPAL™ 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 brewLAP™ 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 has been completed as directed.

BEER SAMPLE PREP and PCR

1. Transfer 25 mL of beer sample to a 50 mL conical tube.
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 250 µL of BUFFER A. Mix until the pellet is no longer visible.
5. Transfer 5 µL from resuspended pellet in 50 mL conical tube generated in step 4 to brewLAP™/vinoPAL™ PCR Tube.
 - a. Open brewLAP™/vinoPAL™ PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
6. Place brewLAP™/vinoPAL™ PCR Tube into IS PCR Thermocycler, select "brewLAP" program and press "START RUN", as directed by the Thermocycler User Guide.
7. Upon completion of "brewLAP" program, press "STOP RUN," and proceed to Cassette Sample Analysis section step 1.

COLONY SAMPLE PREP and PCR

1. Pick and transfer colony into a 1.5 mL microcentrifuge tube containing 500 µL of dH₂O.
2. Mix contents by pipetting sample up and down or by vortexing.
3. Transfer 5 µL of colony re-suspension to brewLAP™/vinoPAL™ PCR Tube.
 - a. Note: Open brewLAP™/vinoPAL™ PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
4. Place brewLAP™/vinoPAL™ PCR Tube into IS PCR Thermocycler, select "brewLAP" program and press "START RUN" as directed by the Thermocycler User Guide.
5. Upon completion of "brewLAP" program, press "STOP RUN", and proceed to Cassette Sample Analysis section step 1.

CASSETTE SAMPLE ANALYSIS

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

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.

MSDS 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.

APPENDIX 1: ASSAY APPLICABILITY

brewLAP™ has been validated for the detection of *lactic acid-producing microbes* capable of causing spoilage in beer. It is intended to be used on final brewery products and samples from each step in the beer making process.