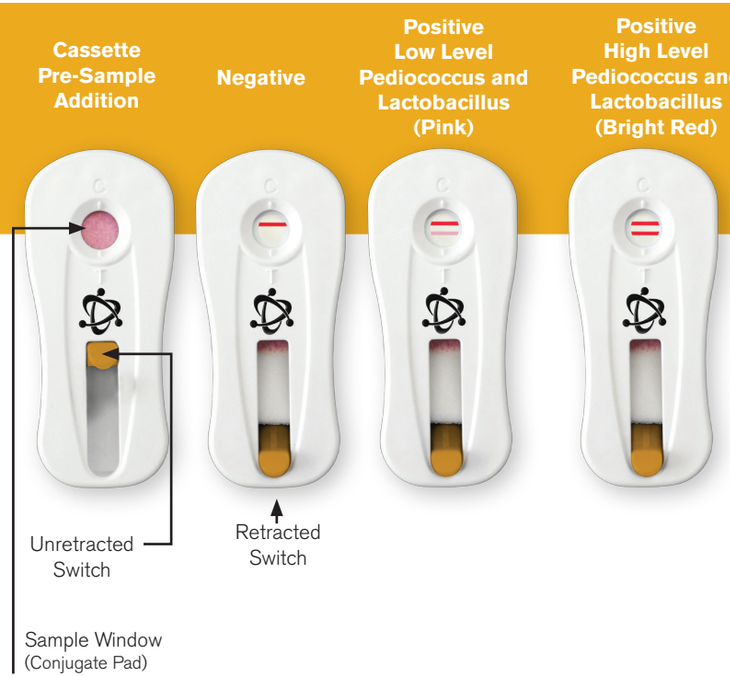
APPENDIX 1: ASSAY APPLICABILITY

brewPAL® has been validated for the detection of *Pediococcus and Lactobacillus* species capable of causing spoilage in beer. It is intended to be used on final brewery products and samples from each step in the brewing process.



APPENDIX 2: RESULTS INTERPRETATION

The control line, as indicated by the letter C on the *brewPAL*® cassette, should always develop. The test line, as indicated by the letter T on the *brewPAL*® cassette, will only develop in the event of a positive sample for *Pediococcus and Lactobacillus* 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. ISO960.2

INVISIBLE SENTINEL®

Phone 215.966.6118 | **Fax** 215.386.3970
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brewPAL Powered by Veriflow®

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

ASSAY PRINCIPLES

brewPAL® is a molecular based assay for the qualitative and quantitative detection of *Pediococcus and Lactobacillus* species capable of causing spoilage in brewery products. 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. *brewPAL*® eliminates the need for gel electrophoresis or fluorophore- based detection of target amplification and provides same day results in under 3 hours. Ultimately, *brewPAL*® provides the specificity and sensitivity of PCR based amplification in a cost-effective and easy-to-use format.

INTENDED USER

brewPAL® is intended for use by personnel familiar with basic sample collection and preparation techniques associated with spoilage organism detection during fermentation and bottling. *brewPAL*® 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 *brewPAL*® PCR Tube – Cat. No. IS0520200
2. IS Buffer A – Cat. No. IS0701
3. IS Buffer B – Cat. No. IS0702
4. IS *brewPAL*® Assay Cassette – Cat. No. IS0121

MATERIALS PURCHASED SEPARATELY

1. IS Lactobacilli MRS Broth – IS0327

MATERIALS NEEDED

1. Invisible Sentinel SimpliAmp PCR Thermocycler – Cat. No. ISTC002
2. Centrifuge compatible with 50 mL conical tubes, capable of 3000 x g
3. Pipettes and tips capable of 5 µL, 200 µL and 250 µL volume transfers
4. 50 mL conical tubes (capable of being centrifuged at 3000 x g)

STORAGE OF MATERIALS

The *brewPAL*® kit components, including cassettes and buffers (Buffer A and B) should be stored at room temperature (20°-25°C). The *brewPAL*® 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 *brewPAL*® 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 *brewPAL*® PCR Tube.
    a. Open *brewPAL*® PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
6. Place *brewPAL*® PCR Tube into IS PCR Thermocycler, select "brewPAL" program and press "START RUN", as directed by the Thermocycler User Guide.
7. Upon completion of "brewPAL" program, press "STOP RUN," and proceed to Cassette Sample Analysis section step 1.

YEAST SLURRY PREP and PCR

1. Transfer 5 mL of yeast slurry to a 50 mL conical tube containing 45 mL of dH_2O. Mix thoroughly by inverting 12-15 times until the mixture is homogenous.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 20 x g.
3. Transfer the top 25 mL of supernatant to a new 50 mL conical tube.
4. Centrifuge 50 mL conical tube generated in step 3 above for 10 minutes at 3000 x g.
5. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
6. Resuspend pellet in 50 mL conical tube with 1 mL of Buffer A. Mix until the pellet is no longer visible.
7. Transfer 5 µL from resuspended pellet in 50 mL conical tube generated in step 6 to *brewPAL*® PCR Tube.
    a. Open *brewPAL*® PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
8. Place *brewPAL*® PCR Tube into IS PCR Thermocycler, select "brewPAL" program and press "START RUN", as directed by the Thermocycler User Guide.
9. Upon completion of "brewPAL" program, press "STOP RUN," and proceed to Cassette Sample Analysis section step 1.

FERMENTATION SAMPLE PREP and PCR

1. Transfer 30 mL of homogenized fermentation sample to a 50 mL conical tube.
2. Centrifuge 50 mL conical tube with sample for 10 minutes at 20 x g.
3. Transfer the top 25 mL of supernatant to a new 50 mL conical tube.
4. Centrifuge 50 mL conical tube generated in step 3 above for 10 minutes at 3000 x g.
5. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
6. Resuspend pellet in 50 mL conical with 1 mL of Buffer A. Mix until the pellet is no longer visible.
7. Transfer 5 µL from resuspended pellet in 50 mL conical tube generated in step 6 to *brewPAL*® PCR Tube.
    a. Open *brewPAL*® PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
8. Place *brewPAL*® PCR Tube into IS PCR Thermocycler, select "brewPAL" program and press "START RUN", as directed by the Thermocycler User Guide.
9. Upon completion of "brewPAL" program, press "STOP RUN," and proceed to Cassette Sample Analysis section step 1.

2X LACTOBACILLI MRS BROTH MEDIA PREP

1. Add 110 g MRS media per 1 Liter dH_2O.
2. Heat/stir until dissolved (~5-10 minutes on heated stir plate).
3. Autoclave rehydrated media at 121°C for 15 minutes and allow to cool to room temperature (20-25°C).
    a. Note: When taken out of the autoclave, MRS will have a cloudy appearance and will be medium amber in color. Avoid leaving in autoclave longer than necessary to prevent over-darkening of media. Cloudiness immediately following autoclaving is normal, and the solution will turn clear as it cools to room temperature.
4. Once 2X MRS has cooled to room temperature, aseptically aliquot 25 mL of 2X MRS into sterile 50 mL conical tubes.
    a. Note: transfer recommended in a laminar flow hood, if available.

ZERO TOLERANCE ENRICHMENT METHOD and PCR

1. Transfer 25 mL of beer sample to a 50 mL conical tube containing 25 mL of 2X MRS broth.
2. Cap conical tube generated in step 2, invert gently, and incubate for 18 hours at 30-32°C.
    a. Note: Leave 50 mL conical tube cap slightly loose (1/4 to 1/2 turn) if the sample contains high yeast counts or if the sample may still be emitting CO_2.
3. Remove conical tube from incubator, cap the tube tightly and centrifuge for 10 minutes at 3000 x g.
4. Decant supernatant from 50 mL conical tube (be careful not to disturb pellet).
5. Resuspend pellet in 50 mL conical tube with 250 µL of Buffer A. Mix until the pellet is no longer visible.
6. Transfer 5 µL from resuspended pellet in 50 mL conical tube generated in step 5 to *brewPAL*® PCR Tube.
    a. Open *brewPAL*® PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
7. Place *brewPAL*® PCR Tube into IS PCR Thermocycler, select "brewPAL" program and press "START RUN", as directed by the Thermocycler User Guide.
8. Upon completion of "brewPAL" 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 dH_2O.
2. Mix contents by pipetting sample up and down or by vortexing.
3. Transfer 5 µL of colony re-suspension to *brewPAL*® PCR Tube.
    a. Note: Open *brewPAL*® PCR Tube only when adding sample and promptly close after, to avoid cross-contamination between tubes.
4. Place *brewPAL*® PCR Tube into IS PCR Thermocycler, select "brewPAL" program and press "START RUN" as directed by the Thermocycler User Guide.
5. Upon completion of "brewPAL" 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 *brewPAL*® PCR Tube.
2. Transfer entire contents (200 µL) of *brewPAL*® PCR Tube directly to *brewPAL*® cassette sample window with pipette. A separate *brewPAL*® cassette must be used for each *brewPAL*® PCR Tube.
3. Allow *brewPAL*® cassette to develop for 2 minutes ± 15 seconds.
4. Add 4 drops of BUFFER B directly to each *brewPAL*® cassette sample window.
5. Allow *brewPAL*® cassette to develop for 1 minute ± 15 seconds.
    a. Note: *brewPAL*® cassette can be developed for up to 120 min before proceeding to step 6.
6. Retract *brewPAL*® cassette switch and record results.
    a. The appearance of one red line (control) in the *brewPAL*® cassette sample window indicates a negative result.
    b. The appearance of two red lines (control and test) in the *brewPAL*® cassette sample window indicates a positive result.
        i. Test line intensity indicates quantitative levels of *Pediococcus and Lactobacillus* species.

CUSTOMER SERVICE

Invisible Sentinel customer service and technical assistance can be reached Monday-Friday between 9 AM and 5 PM 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

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

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