Exponential Concentration of Spoilage Organisms in Carbonated Beverages for Same Shift Detection Using Concentrating Pipette Select System

Application Note

Introduction:

Microbiological testing is crucial at every stage of beverage production, such as manufacturing, winemaking, or brewing, to maintain product quality. Rapid microbiological analysis techniques like qPCR can enhance spoilage detection reliability in beverages while cutting labor costs and product delays. However, these methods have limited usefulness due to their small analysis volumes. To address this issue, overnight pre-enrichment is commonly employed, but that negates the time-saving advantage of rapid analysis.

InnovaPrep has created a rapid "mechanical concentration" method to remove the need for culture-based enrichment steps. This technique utilizes a one-time-use high-flow membrane filter and an innovative elution process, enabling quick, easy, and user-friendly concentration of spoilage organisms in beverages.

The InnovaPrep Concentrating Pipette System quickly concentrates microorganisms, achieving up to 10,000 fold concentration from liquid samples. The system filters the sample and then employs a Wet Foam Elution method to quickly recover the microorganisms into a much smaller volume. The system's ease of use and ability to deliver exceptionally high concentration factors and simultaneous clean buffer exchange make this system an ideal solution for trace analysis of spoilage organisms.

The Concentrating Pipette System is appropriate for bacteria, molds, spores, and viruses. The system provides rapid *concentration of spoilage organisms in carbonated beverages* and is suitable for a variety of applications including food safety and outbreak investigations.

Materials Required:

- **Instrument: CP Select™ and CP Select™ User Manual**
- **InnovaPrep Be Flat™ Degassing Jar** Item #HC08674
- **Consumables:**
  - **Elution Buffer: Tris** Item # HC08001
  - **Concentrating Pipette Tips (CPTs):** (see Tip Selection Guide for more information)
    - **0.45 µm CPTs –** Item # CC08018-10

SAFETY:

Due to the potential presence of infectious pathogens in samples, users should work with their organization's occupational safety team to ensure that methods and safety measures are appropriate and approved. Unless working with samples known to be non-infectious, InnovaPrep recommends that CP Select™ operations be

STEP 1 – Choose Elution Buffer

- Select from the below elution buffer formulations, according to your method of analysis:

  Classical Culture- HC0800- 0.075% Tween 20 PBS

  Rapid Methods- HC08001- 0.075% Tween 20 Tris

STEP 2 – Decontaminate Sample

- It is necessary to decarbonate beverages before processing on the Concentrating Pipette as CO_2 in the liquid interferes with the flow sensor and tends to blind the membrane. InnovaPrep has developed the **Be Flat™ Degassing Jar**, which incorporates a unique, patented design to rapidly degas carbonated beverages.
- To degas, pour the room temperature beverage into the Be Flat™ Degassing Jar rapidly from a few inches (several centimeters) above the jar. This will encourage the release as much CO_2 as possible. Place jar in refrigerator for 10 minutes.
  - Note: It is important to evaluate the optimal time for degassing as each beverage differs. Some beverages may not require a refrigeration step, while others may take up to 20 minutes for degassing.

STEP 3 - Concentration

- Set up the CP Select™ as instructed in Section 4 of the User Manual.
- Insert a CPT and select the HOLLOW protocol from the menu.
- Lower the CPT into the sample.
- Press "Start Run" on the user screen. When the entire sample has been processed the CP Select™ will stop.
- Place a clean final sample container under the CPT. The menu screen will prompt you to press "Elute".
- Press "Elute". The sample will dispense from the pipette tip into the sample container. The sample is ready for subsequent sample preparation and analysis steps.

  - *Note:* ***Process volumes will vary according to beverage type, temperature, and amount of carbonation remaining in the sample.***

STEP 3 – Analysis Method

- Samples are suitable for a variety of culture and rapid methods, including digital PCR/qPCR, digital RT-PCR/RT-qPCR, NGS, Lateral Flow Assays, etc. The samples concentrated by the CPT are now ready for analysis and any further downstream processing steps.
- Store pre-concentrated or post-concentrated liquid samples at 4 °C.

Notes:

Concentrating Pipette Tips come in a variety of pore sizes. Users can access the Consumable Selection Guide on the InnovaPrep website to determine which tip will be optimal for their target of interest and application.

The final eluate can be analyzed using classical culture or rapid molecular methods of your choice.

Pooling and concentrating multiple samples into a single concentrated sample can be useful for certain applications, such as environmental monitoring, as it can lower analysis costs and improve method sensitivity. When using this approach, it is important to note that identification of the exact location of a positive result may require additional sampling or analysis of reserved aliquots.

*Please check our website for the most current methodology as updates are published periodically.

References:

2018 - A Rapid and Efficient Sample Preparation Method for Spoilage Organisms Detection in American Lager Beer

2019 - Concentration of Lactobacillus brevis from Experimentally Infected American Lager Beer and Wine by InnovaPrep's Concentrating Pipette and Degassing Jar

2019 - Recommendations for rapid concentration of spoilers in Hazy Beers using the Concentrating Pipette Select

2019 - Concentrating Pipette for Improved Detection and Same Shift Results - Rapid Microbiology News

US Patent 10,953,350

EPO Patent 3,612,036