Method Development Guidance for Reducing Incubation Time for Homogenized Foods

Application Note

Introduction:

The InnovaPrep Concentrating Pipette Select (CP Select) is an automated, rapid micro-particle concentrator developed for general microbiology use. The system performs mechanical enrichment as a front-end to rapid microbial detection in minutes, eliminating the need for centrifugation or enrichment in many sample types- or greatly reducing enrichment times for more difficult matrices such as food homogenates.

The one-pass method uses tangentially loaded dead-end filtration to capture micro-organisms in high-flow hollow fiber membrane filters within the InnovaPrep CP's single-use tip.
After the sample has been processed and the particles have been trapped, InnovaPrep's patented Wet Foam Elution™ process is employed to recover the particles from the membrane into a very small user selectable final volume.

Concentrating homogenized food samples is one application of interest for this technology and holds promise for substantially reducing enrichment times, often in half. The concentrated sample pairs directly with classical and modern analytical methods commonly used in food diagnostics today. The following guidance offers a starting point for users exploring new methods to reduce time to detection for same shift results and as a means for reducing costs associated with holding product, especially perishable foods that require refrigeration.

Method Development Guideline:

This guidance follows the FDA's Bacteriological Analytical Manual (the BAM) with alteration to the recommended enrichment time for use with the InnovaPrep CP. The BAM is FDA's preferred laboratory procedures for the detection in food and cosmetic products of pathogens (bacterial, viral, parasitic, plus yeast and mold) and of microbial toxins. The manual is available online: http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm.

It is important to note that even if only a portion of the sample can be concentrated efficiently, a significant concentration factor will be produced that will aid any detection method used.

Please also note, the following protocol is not an AOAC standard. AOAC standards are used globally to facilitate public health and safety. For AOAC certification, a validation study must be performed for each specific food type/sample matrix. Once a protocol is validated for reduced enrichment times for your desired matrix the AOAC approval process can be initiated.

Study Design:

Designing a spike study for your matrix to determine limit of detection is the first step in determining how far you can reduce enrichment time using the CP Select. For study design guidelines, consult the AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces.

Materials Required:

- **Instrument: CP Select**
- **Consumables:** Click here for our Consumable Selection Guide
    - **Elution Buffer:** Item # HC08001 (Tris) or # HC08000 (PBS)
    - **Concentrating Pipette Tips (CPTs):**
        - **Ultrafilter CPTs** – Item # CC08003-10
        - **0.05 µm CPTs** – Item # CC08020-10
        - **0.2 µm CPTs –** Item # CC08022-10
        - **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 performed

STEP 1 – Preparing the Sample

- Collect and prepare food homogenate as directed in the BAM protocol.
- Combine the appropriate ratio of sample to enrichment media as directed in the BAM (example: 25g homogenate to 225mL media).
- Add materials to filtered stomacher bag and process normally
- Incubate at the recommended temperature for a reduced period. 2/3 or half the normally recommended time is a good starting point.
- Remove from incubator or water bath.
- Pass approximately 50 mL of the incubated sample though the filter section of the Bag Filter.

STEP 2 – Concentration

- Set up the Concentrating Pipette Select as instructed in Section 7 of the CP Select User Guide.
- Insert a Concentrating Pipette Tip (CPT) and select a menu protocol as instructed in Section 8 of the CP Select User Guide for the chosen CPT type.
- Lower the CPT into the sample.
- Press "Start Run" on the user screen. When the entire sample has been processed the CP 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.

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 cleanroom 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.

***Hints and Troubleshooting**: A technical report entitled "Reduced Incubation for Detection of Escherichia coli O157:H7 in Experimentally Infected Spinach Samples using the InnovaPrep Concentrating Pipette" is available by request.*

References: