Concentration of Bloodborne Pathogens in Whole Blood

Application Note

Introduction:

The InnovaPrep Concentrating Pipette (CP Select™) is an automated, rapid micro-particle concentrator developed for general microbiology use. The system performs rapid "mechanical enrichment" as a front-end to rapid microbial detection, replacing older, slower, labor-intensive methods such as centrifugation or culture-based enrichment steps. The one-pass method provides sample volume reduction and removal of matrix-associated inhibitors for modern analysis methods such as qPCR.

The concentration process uses tangentially-loaded, dead-end filtration to capture particles on porous membrane filters within the InnovaPrep CP's single-use tip. After the sample has been processed and the pathogens have been trapped, InnovaPrep's patented Wet Foam Elution™ process is employed to wash the pathogens off of the membrane surface into a very small liquid volume.

Concentrating blood product samples for research is one application of interest for this technology that holds promise by delivering concentrated samples of blood-borne bacteria to rapid detection methods such as real-time polymerase chain reaction systems. A simple protocol for preparing blood prior to concentration using the InnovaPrep CP is provided below. Many users have continued method development beyond this protocol with excellent success.

The following preparation method can render whole blood that is "filterable" through membrane filters that are used in the InnovaPrep CP. Because complex clinical samples can foul membrane filters, a means for removing or passing these components through the filter tips must be implemented. The lysis protocol uses a non-ionic surfactant to lyse red blood cells and then an enzyme to further break down the red blood cell components to the point that they will readily pass through the membrane filter in the Concentrating Pipette Tip (CPT). Note that Universal Precautions are required for working with blood and blood products.

Materials Required:

- **Instrument: CP Select™ - InnovaPrep**
- **Consumables:**
  - **Elution Buffer: Tris** Item # HC08001 or HC08000
  - **Concentrating Pipette Tips (CPTs):** (see Tip Selection Guide for more information)
    - **0.45 µm CPTs-** Item #CC08018-10
    - **0.2 µm CPTs –** Item # CC08022-10
- **Vacutainer™ Glass Blood Collection Tubes with SPS -** Becton Dickenson Item #364960
  - Contains citrate dextrose additives for anticoagulation and preservation
- **Blood rocker**
- **Lysis buffer (A solution of 0.01 M Na2PO4/1% Tween 20 buffer - adjusted to a pH of 9.0-11.5)**
- **≥500 LAPU/g Protease from Aspergillus oryzae -** Sigma-Aldrich, Item #P6110
- **Shaking water bath**

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

Procedure:

STEP 1 - Sampling Method

The following blood lysis method was demonstrated to make it possible to process volumes of 10 mL or more of human whole blood in the InnovaPrep CP to a final concentrate volume of approximately 200 µL for subsequent analysis.

- Collect 5mL blood directly into pre-weighed, sterile BD SPS Vacutainer with SPS
- Place the filled vacutainers onto a blood rocker to reduce clotting.
- Prepare a solution of 0.01 M Na2PO4/1% Tween 20 lysis buffer.
- Adjust the pH of the lysis buffer to the range of 9.0-11.5. Raising the pH to this range reduces the amount of time needed for an effective reaction to take place.
- Using the stock 500 LAPU/g protease prepare a 50 LAPU/g solution.
- Add 24 mL of pH adjusted lysis buffer and 4 mL of 50 LAPU/g protease to a specimen cup.
- Add the volume of either one or two vacutainers of blood to the specimen cup (up to 10 mL).
- Place the specimen cups into a shaking water bath.
- Allow to incubate at 37° C for a period of 45 to 65 minutes. At the beginning of the incubation period the blood/buffer mixture will be an opaque blood-red fluid. Toward the end of the incubation period the sample quickly changes to a clear, dark reddish-brown color. Once the color change occurs, allow incubation to continue for an additional 5 to 10 minutes to ensure that complete lysis has occurred. Removing the sample too quickly may result in filter fouling and premature stoppage of the concentration run.

STEP 2 - Concentration

- Set up the Concentrating Pipette (CP) as instructed in Section 4 of the CP Select User Guide.
- Insert a Concentrating Pipette Tip (CPT) and select a menu protocol as instructed in Section 5.2 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 & TROUBLESHOOTING GUIDE

A technical report entitled “Blood Methods Development Research” is available by request.

References:

2018 - Inactivation of Ebola virus and Middle East respiratory syndrome coronavirus in platelet concentrates and plasma by ultraviolet C light and methylene blue plus visible light, respectively

2006 - Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products