INNOVAPREP®

CONCENTRATING PIPETTE SELECT
WASTEWATER APPLICATION NOTE
Revision B

Concentrating Pipette Select™

INTRODUCTION

The InnovaPrep Concentrating Pipette Select™ (CP Select) is an automated, bio-concentration device for modern microbiology. The CP Select uses dead-end filtration with single-use Concentrating Pipette tips (CPT) to capture microorganisms from the fluid sample matrix. A novel process called Wet Foam Elution is then used to recover the micro-organisms into a small sample volume. The system's ease of use and ability to deliver exceptionally high concentration factors make it an ideal approach for concentration of SARS-CoV-2 in wastewater (WW). This application note provides considerations specifically related to detection of SARS-CoV-2 in wastewater influent (InnovaPrep can also provide guidance on wastewater effluent method development). SARS-Cov-2 wastewater monitoring is a new and evolving application, and InnovaPrep is continuing method development. We recommend referencing the latest revision of this document for the most current methodology.

METHOD

Safety:

Due to the potential presence of infectious SARS-CoV-2 in wastewater 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 in a biosafety cabinet. One source of information is published by the U.S. CDC: Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

MATERIALS REQUIRED:

· InnovaPrep Concentrating Pipette Select
· CP Elution Fluid Can- Tris (HC08001)
· Concentrating Pipette Tip (CPT) Ultrafiltration-CC08003 (More turbid wastewater samples may require 0.05µm-CC08020)
· DISCOUNTED CPTs NOW AVAILABLE IN BULK- NON-STERILE

· Precentrifugation tools
   · 7,000-10,000 g Centrifuge (eg. Eppendorf 5804 or similar)
· Tween 20 for sample addition

PROCESS

Step 1 – A CPT should be selected based on preliminary evaluation of your wastewater matrix and your selected pretreatment method. Both CPT types listed below have been shown to capture SARS-CoV-2 in wastewater.
   · Ultrafiltration PS Hollow Fiber Concentrating Pipette CPTs (CC08003) (slightly higher efficiency)
   · 0.05 µm PS Hollow Fiber Concentrating Pipette CPTs (CC08020) (slightly faster processing)

Step 2 – Preparing the Influent wastewater

   1. Prepare a 5% Tween 20 stock solution
   2. Add the prepared Tween 20 to the starting Influent wastewater sample at 1:100 (e.g. 1 mL addition to 100 mL influent wastewater; 40-50 mL sample volume is common when using ultrafiltration CPTs while 100 mL sample volume is common when using 0.05 µm CPTs). (Tween 20 has been shown to significantly increase SARS-CoV-2 recovery from wastewater)
   3. Centrifuge wastewater sample for 10 minutes at 7,000 to 10,000 g (optimize g-force and time for your matrix)

Step 3 – Concentration
   1. Setup the CP Select as instructed in Section 4 of the CP Select User Guide.
   2. Following the instructions provided in Section 8 of the CP Select User Guide, set up a Custom Protocol using the Advanced Options as shown on the next page.
   3. Insert CPT and select menu protocol as instructed in Section 5.2 of the CP Select User Guide.
   4. Lower CPT into the sample.

Protocol Name	Valve Open ms	Pulse	Foam Factor	Valve Closed (ms)	Valve Start (sec.)	Flow End (sec.)	Flow Min Start (sec.)	Ext Delay (sec.)	Pump %	Ext Pump Delay (sec.)
COVID-WW	800	1	10	100	3.0	10	40	3	25%	1

This protocol is designed to deliver sufficient concentrated sample volume to perform two nucleic acid extractions with standard kits (one for an initial run and one as an archive sample). Sample concentrate volumes should be optimized to minimize the mismatch between this volume and the nucleic acid extraction protocol input volume. The concentrate volume can be increased by increasing the valve open time and/or pulse count (for open times above 800 ms it is suggested that 2 pulses be used). A pump setting of 25% has been demonstrated to enhance recovery and is suggested for this application.

5. Press the "Start Run" on the user's screen. When the entire sample has been processed the CP Select will stop.

6. Place a clean final sample container under the CPT. The menu screen will prompt you to press "Elute."

7. Press "Elute." The sample will dispense from the CPT into the sample container. The sample is ready for subsequent sample preparation and analysis steps.

Step 4 – Sample extraction and analysis method of choice (reverse transcription ddPCR or reverse transcription qPCR)

PERFORMANCE

Influent LOD: 1 copy/ 1mL when processing 100 mL of WW

Clarified Effluent LOD: 5 copies / 100mL when processing 1000 mL of WW. (For effluent samples, InnovaPrep Large Volume Concentrator (LVC)Kit item #CC01116-T) is recommended as a first concentration step, in conjunction with the CP Select. LVC Kits are available on the InnovaPrep website.)

TROUBLESHOOTING

1. Unable to process the entire sample

(Instrument shuts off in less than 1 min., instrument shuts off prematurely.)

Troubleshooting / CPTs

· Ensure CPT is fully inserted into the CP Select before processing samples (user should feel CPT "click" into place).

· When processing samples make sure **CPT is fully submerged** until the entire sample is processed. Do not pull CPT from the liquid during concentration, any air ingress into the CPT will cause it to "lock-up" after being wetted.

· Make sure the waste / permeate line is fully inserted/plugged into the CP Select (should click into place).

· Utilize a more rigorous pretreatment method. Recommendation: Spin sample for **10 min at 10,000 g** or **prefilter using** a 0.45 µm minimum pore size, 47 mm minimum diameter PES or similar hydrophilic membrane filter w/ dull/matte side up. Larger membrane filter pore sizes and filter diameters will reduce losses of SARS-CoV-2 with only minimal negative effect on CP Select filterability.

· Reduce the shutdown sensitivity of the CP Select: Create a Custom Protocol, increase the Flow End to 10.0, Save Protocol (*Note: go to System Settings and turn on "Advanced". This will let you see and change all variables, including Flow End*)

· Ensure CP Select is properly maintained. Be sure to *grease the O-rings* by applying a light coat of vacuum grease to the maintenance CPT.

· Make sure you have selected the proper filter for your application. Both 0.05 micron and Ultra CPTs have been shown to effectively capture SARS-CoV-2. The 0.05 CPTs will enable processing of slightly more challenging samples.

2. Efficiency is lower than desired

Troubleshooting / CPTs

· Use wastewater (not deionized water). In some cases, poor results have been experienced for virus spiked into deionized water. InnovaPrep is working to improve recovery for viruses in deionized water.

· Add surfactant to the starting sample. The addition of Tween 20 to the starting sample will often increase recovery. A final Tween 20 concentration of 0.05% to 0.1% in the sample is common although higher concentrations may be beneficial in some instances. Contact InnovaPrep for assistance and recommendations.

· Reduce the pump speed to 25%: Create a Custom Protocol and adjust the pump % variable. (Note: go to System Settings and turn on "Advanced". This will let you see all variables).

· Increase your elution volume. Use the Ultrafilter setting, elute more than once, or create a Custom Protocol and increase the "Valve On Time" and/or add multiple pulses (*Note: although the efficiency can be increased in this way, Concentration Factor may be reduced due to the larger final volume*).

3. Concentration Factor is lower than desired

Troubleshooting / CPTs

· Reduce the Elution Volume, create a custom protocol, Reduce "valve on time" and/or "Pulses" to achieve a smaller elution volume.

· Increase the starting sample volume.

· Increase efficiency using the items suggested Section 2, above.