

Virus Concentration for Optimal Infectivity - InnovaPrep® Concentrating Pipette Select Comparison Study

Revision B*

This report contains the results of brief comparison testing performed by Lawrence Livermore National Laboratory (Livermore, CA) of two concentration methods: The InnovaPrep Concentrating Pipette device using two of the system's available Concentrating Pipette Tips (CPTs), and EMD Millipore Amicon Ultra 15 (10KD) centrifugal filters for virus concentration with three virus sample matrices. It includes information that can be used by any laboratory to concentrate enveloped viruses with conserved infectivity using a custom Dulbecco's modified Eagle medium (DMEM).



Introduction:

InnovaPrep provided a CP-150 Concentrating Pipette base station to LLNL for this testing. The Concentrating Pipette Select (CP Select) is the improved, next generation instrument that replaced the CP-150 in 2018. The CP Select is an automated, rapid micro-particle concentrator that uses tangentially-loaded dead-end filtration to capture particles onto the inside surfaces of hollow fiber membrane filter elements within the Concentrating Pipette's single-use tip. After the sample has been processed and particles have been trapped in the filter, InnovaPrep's patented Wet Foam Elution™ process is initiated by the press of a button to rapidly elute the particles from the inside bores of the filter elements into a small volume (~250 µL) of clean buffer solution or viral culture media. The system accommodates a variety of Pipette Tips based on pore size. For this testing two Tip types were used: The 0.05 µm and the Ultrafilter tip.

The Amicon Centrifugal Filter Units are centrifuge tubes that contain a module made from an Ultracel regenerated cellulose membrane in a range of molecular weight cut-offs. When the sample is spun in a centrifuge, the particles are collected on the membrane. To recover, the inner membrane module within the tube is inverted, spun again and the concentrated sample is deposited in the bottom of the centrifuge tube. Amicon Ultra units are available in 0.5 mL, 4mL & 15ml volumes. The 15 mL tubes with the 10 kD cut-offs were used in this testing.



System Specification Comparison

	Concentrating Pipette 0.05um Tips	Concentrating Pipette Ultrafilter Tips	Amicon Ultra 15 10 KD
Active Membrane area	82 cm ²	82 cm ²	7.6 cm ²
Maximum initial sample volume	up to 3 liters (matrix dependent)	up to 500 mL (matrix dependent)	15 mL
Typical final concentrate	200 µL to 1 mL User selectable range. Particles in clean buffer	200 µL to 1 mL User selectable range. Concentrate in clean buffer	200 µL Concentrate in sample matrix
Processing time (concentration + elution)	up to 90 mL/min. followed by 30 second sample recovery.	up to 25 mL/min. followed by 30 second sample recovery.	Centrifuge 10–30 minutes depending on the NMWL of the device used. (in swinging bucket rotor at 4000 x g. Spin times: 3K (40 min); 10K and 100K (20 min); 30K (10 min); 50K (15 min). Followed by 2 min sample recovery.
Required equipment	Concentrating Pipette base station.	Concentrating Pipette base station.	Centrifuge with fixed angle rotor that can accommodate 1.5 mL microcentrifuge tubes

Note: Since completion of this testing, the total membrane count in the tips has increased by 10%, which correlates to a 98cm² nominal surface area, improving both the flow rate and reduced membrane fouling.

The ability to concentrate viral stocks from supernatants is often needed to achieve high enough viral titers to perform in vitro or in vivo experiments. This process can be laborious and time consuming often requiring the use of an ultracentrifuge or specialized centrifugal filters. To determine if there was a more efficient process of concentrating virus from supernatants, the original CP-150 Concentrating Pipette system was compared to the Amicon Ultra-15 10KD centrifugal filter unit, resulting in the below data.

Coxsackievirus is an enterovirus, belonging to the Picornavirus family. It is a non-enveloped single-stranded positive-sense RNA virus. Sindbis virus (SINV) is an alphavirus belonging to the Togaviridae family. SINV is an enveloped single-stranded positive-sense RNA virus. Dengue virus is a flavivirus belonging to the Flaviviridae family. DENV is an enveloped single-stranded positive-sense RNA virus.

The improved Concentrating Pipette Select (CP Select) can be used to perform virus concentration using the following method. DMEM elution fluid is available by special order through InnovaPrep Customer Service.

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 in a biosafety cabinet.

Materials Used:

- **Instrument: CP Select**
- **Consumables:**
 - **Elution Buffer: Prototype** DMEM Elution Buffer
 - **Concentrating Pipette Tips (CPTs):** (see Tip Selection Guide for more information)
 - **Ultrafilter CPTs** – Item # CC08003-10
 - **0.05 µm CPTs** – Item # CC08020-10

Procedure:

For the original LLNL demonstration, 15mL of viral supernatant was used, as that is the maximum volume allowed for the Amicon tubes, though the InnovaPrep system has the ability to process a much larger volume. Recovered viral concentrates and supernatant were serially diluted and used to infect BHKs to determine viral titers (pfu/mL). The total virus in the supernatant was calculated (pfu/mL) x 15mL which was then set at 100%. Viral recovery was determined by dividing the total [virus] in the filtrate, (pfu/mL) x final volume, then dividing by the starting Total in the supernatant.

The Amicon tubes were centrifuged for 40 minutes. The 0.05µm Pipette tips took 3 minutes to filter the 15 mL sample. The Ultrafilter Pipette tips took 6 minutes to filter the 15 mL sample.

CP Select Concentration Procedure

1. Set up the CP as instructed in Section 4 of the CP Select User Guide.
2. Insert a CPT and select a menu protocol as instructed in Section 5.2 of the CP Select User Guide for the chosen CPT type as well as comparing to the data table below.
3. Lower the CPT into the sample.
4. Press “Start Run” on the user screen. When the entire sample has been processed the CP will stop.
5. Place a clean final sample container under the CPT. The menu screen will prompt you to press “Elute”.
6. 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. A second elution can also be performed if desired. The first elution is likely to contain the highest concentration of virus; however, additional virus may be collected in a second elution.

Results

Coxsackievirus	InnovaPrep 0.05µm Tips	InnovaPrep UltraFilter	Amicon Tubes
Starting volume	15mL	15mL	15mL
Time	3 min.	6 min	40 min.
% Recovery	98%	70%	65%
Sindbis Virus			
Starting volume	15mL	15mL	15mL
Time	3 min.	6 min	40 min.
% Recovery	62%	60%	56%
Denv2			
Starting volume	15mL	15mL	15mL
Time	3 min.	6 min	40 min.
% Recovery	15%	45%	65%

Notes:

Prototype Elution Buffer: For this testing InnovaPrep provided a custom prototype elution buffer, to conserve the infectivity of the virus. This fluid is available as a custom order by contacting InnovaPrep Customer Support.

- DMEM fluid was loaded into the usual InnovaPrep elution fluid canister and charged with nitrous oxide to produce wet foam for system operation.

Viscous media matrices may require the need for optimized menu settings on the CP Select. The standard *Hollow* Protocol was used in this test series and is recommended as a starting point. If the system shuts down prematurely using these settings, the following optimizations may prevent premature shutdowns. Please refer to The CP Select User Guide, section 8 for more information on menu settings.

Protocol Name	Valve open ms	Pulse	Foam Factor	Valve Closed ms	Flow Start sec.	Flow end sec.	Flow min start sec.	Ext Delay sec.	Pump %	Ext pump delay sec.
	770	1	10	100	3.0	10	40	3	25%	1

Conclusions:

This testing showed that concentration of virus is feasible using the Concentrating Pipette. Infectivity was better preserved when using the DMEM elution fluid as compared to the standard elution fluid.

Note: For the specific evaluation above, the input sample volumes and final concentrated volumes were kept the same for each concentration method for purposes of comparison. However, the Concentrating Pipette is capable of processing larger volumes than the Amicon tubes and thus is capable of generating even higher concentration factors.