Virus Concentration Comparison Testing - InnovaPrep® Concentrating Pipette, an Automated Benchtop Concentrator, and EMD Millipore Amicon® Ultra-15 Centrifugal Filter Units

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Subject: Comparison Study

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 single-use Concentrating Pipette Tips (CPTs), and EMD Millipore Amicon Ultra 15 (10KD) centrifugal filters for virus concentration with three virus sample matrices.

Introduction

InnovaPrep (Drexel, MO) provided a CP-150 Concentrating Pipette base station to LLNL for this testing. The Concentrating Pipette is an automated, rapid micro-particle concentrator that uses dead-end filtration to capture particles onto the surface of a porous membrane filter within the Concentrating Pipette's single-use tip. After the sample has been processed and particles have been trapped on the filter, InnovaPrep's patented Wet Foam Elution™ process is initiated by the press of a button to rapidly elute the particles from the membrane surface into a volume few drops of clean buffer solution. 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 Ultafilter ~ 150 KD pore size cut-off.

The Concentrating Pipette Select™

Figure 2 EMD Millipore Amicon Tube

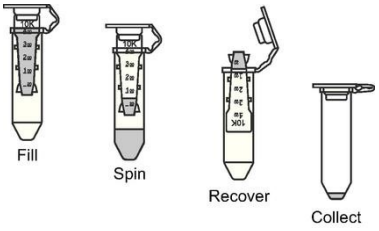

Figure3 Amicon Tube Protocol

The Amicon Centrifugal Filter Units are centrifuge tubes that contain a module made from a high recovery 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
Per sample cost	$25	$25	$11

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 is a more efficient process of concentrating virus from supernatants we evaluated the CP-150 Concentrating Pipette base station against the Amicon Ultra-15 10KD centrifugal filter unit.

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.

Protocol

For this experiment 15mL of viral supernatant was used, 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.

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:

Custom elution foam: For this testing InnovaPrep provided a custom elution fluid, to conserve the infectivity of the virus.

- DMEM fluid containing HEPES, sodium bicarbonate, L-Glutamine, and glucose.
- 1X level of triple antibiotic/antimycotic solution, with a final concentration of 100 units/mL penicillin,100 µg/mL Streptomycin, and 0.25 µg/mL Fungizone® Antimycotic 0.001% BSA
- The fluid was loaded into the usual InnovaPrep elution fluid canister and charged with nitrous oxide to produce wet foam for system operation.

InnovaPrep System shut-off: A system shut down issue was encountered with the Concentrating Pipette due to the flow sensor in the device failing to see matrix flow at start-up. InnovaPrep confirmed this is an issue with thick or viscus matrices with the smaller pore size tips. When sample flow does not reach the sensor within the default 20 seconds the device will stop as though the run is complete so the user must press 'start' again. A menu setting (*flow buffer delay*) can be adjusted up to address this somewhat but InnovaPrep indicated system improvements are underway to solve this. During testing with the 0.05 tips, shut off occurred once at start-up but once restarting had no issues processing the supernatant. The ultrafilter tip had a similar problem pausing 3 times at start-up requiring the need to press 'start' each time.

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. For this testing, the input sample volumes and final concentrated volumes were kept the same for each concentration method. The Concentrating Pipette is capable of processing larger volumes than the Amicon tubes, and should thus be capable of generating higher concentration factors. Future work may include running qPCR to compare the concentration efficiency for molecular detection methods.