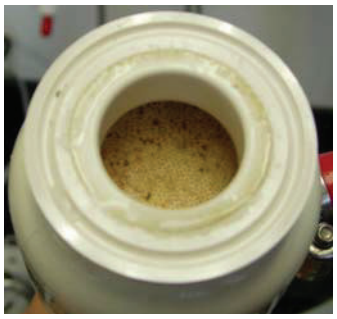
Tangential Flow Filtration

Rohwer Lab
2005

More details are found on the Notes pages – including catalog numbers and vendor details.

This guide shows the basic information for setting up a TFF system

Catalog numbers and prices are current as of July 2005



TFF Basics

In tangential flow filtration (TFF), the sample is run parallel to the filter. Particles that are smaller than the pore size are pushed out through the holes (the filtrate). TFF is most effective when a reservoir is used so that the sample can be recycled through numerous times.

We use 2 sizes of filters. All the filters are from Amersham Biosciences (1-800-526-3593):

Large filters: for concentrating 10 or more liters down to ~1 liter

Large 0.2 μm filter: CFP-2-E-9A ($1306)

Large 100 kD filter: UFP-100-C-9A ($1306)

Medium filters: for concentrating up to 10 liters to a final volume of ~100 ml

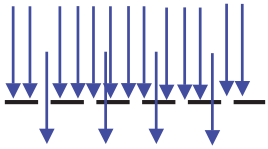
Medium 0.2 μm filter: CFP-2-E-4A ($459)

Medium 100 kD filter: UFP-100-C-4A ($444)

The upper left-hand picture is of the large sized filters – you can see the straw-like filters inside.

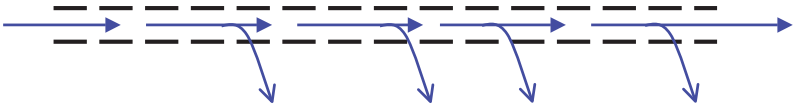
The input can go in either end of the filter, the retentate comes out the opposite end. The filtrate comes out the 2 side outlets – tubing can be attached to both of these, or one can be covered so you only use one filtrate output.

Impact Filter



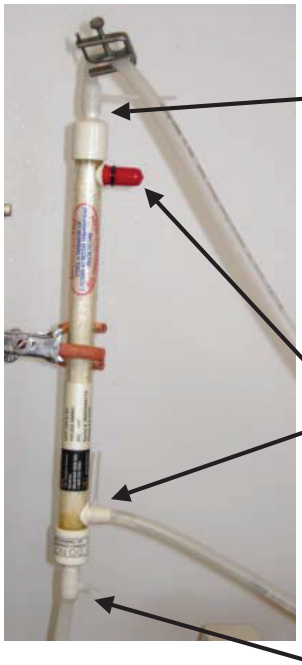
* Sample flows perpendicular to filter
* Small surface area, clogs easily

Tangential Flow Filter



* Sample flows parallel to filter, sample is recycled numerous times through a reservoir
* Large surface area – can filter larger volumes without clogging

Tangential flow filtration allows you to filter large volumes and concentrate them into smaller volumes



Retentate (larger than filter pore size)

•With the 0.2 μM filter, the retentate contains bacteria, protists, and large particles

•With the 100 kD filter, the retentate contains viruses, bacteria, protists, and large particles

Filtrate (smaller than filter pore size)

•With the 0.2 μM filter, the filtrate contains viruses

•With the 100 kD filter, the filtrate is sterile water

Input

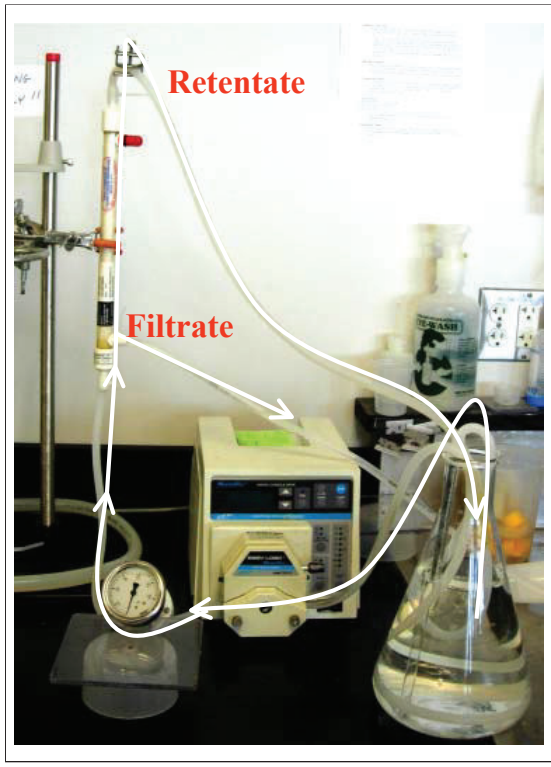
The medium filter is shown here. The medium and large tangential flow filters work in the same way. The main difference is in the amount of surface area, which controls how much volume you can filter per time.

The input can go in either end of the filter, the retentate comes out the opposite end. The filtrate comes out the 2 side outlets – tubing can be attached to both of these, or one can be covered so you only use one filtrate output.

With a 0.2 μm filter, the viruses are in the filtrate

With a 100 kD filter, the viruses are in the retentate

For the medium filters, the tubing (size 15) can be attached directly to the TFF. The tubing is secured to the filter using cable ties. The large filters require a tubing adaptor kit – more information in a later slide.



Overview

Example: medium 100 kD filter

WATCH AS SLIDESHOW!

1) Sample runs from reservoir through peristaltic pump

2) Through pressure gauge

3) Into TFF

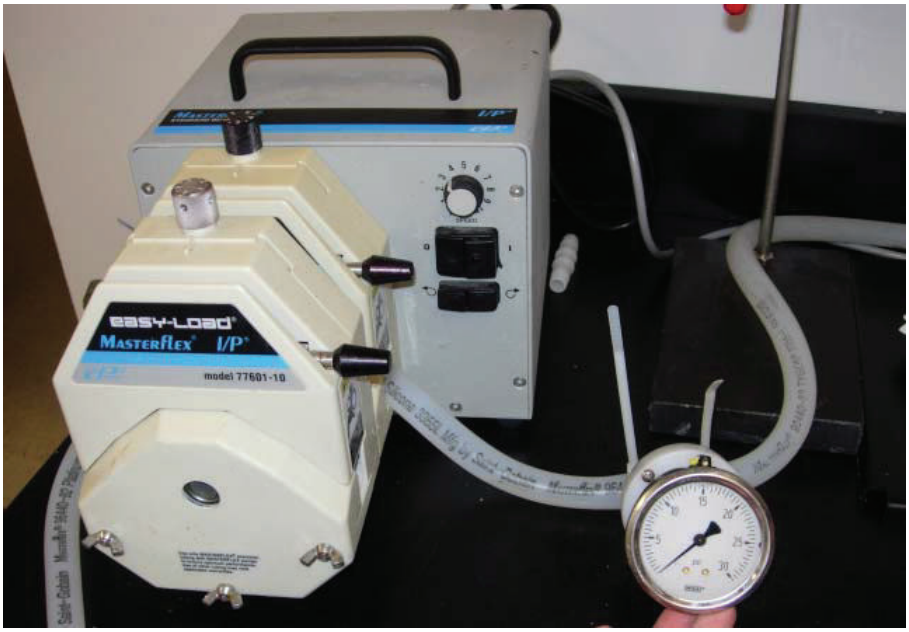
For the 100 kD filter:

* Retentate contains viruses (recycle into reservoir to concentrate)
* Filtrate is sterile water and can be collected or discarded

Our lab setup is shown here. The reservoir can be any container ranging from a beaker to a large trash can. Attaching the filter to a ringstand makes things more manageable, but it doesn't matter if the filter is vertical or horizontal.

In the example shown here, the viruses are being concentrated on a 100 kD filter. The water is being pushed out of the sample while the viruses are retained on the filter and in the retentate. The filter is run until there is very little volume left in the flask. At this point, the pressure on the retentate tube is released, allowing the sample to wash over the filter, freeing any attached viruses. The sample is then concentrated further – with the final step being removal of the input tube which runs air through the filter and forces all remaining sample out of the filter and tubing.

Peristaltic Pump and Tubing



The peristaltic pump, tubing, and pressure gauges are all purchased from Cole Parmer (1-800-323-4340)

Tubing: MASTERFLEX!

Size 82 (large filters): U-96440-82 ($258 for 25 feet)
Size 15 (medium filters): U-96440-15 ($108 for 25 feet)
Size 73 tubing (to adapt medium filters to large pump heads):
U-96440-73 ($196 for 25 feet)

Peristaltic pump:

Size 82 or 73 tubing (large filters):

Masterflex Pump, I/P Brushless Drive: EW-77410-10 ($1475)
Masterflex Pump head, Easy-load PSF/SS: FF-77601-10 ($490)
Mounting hardware for additional pump heads: FF-77601-96 ($22)

Size 15 tubing (medium filters):

Masterflex Pump, L/S Easy-load: FF-77521-40 ($865)
Masterflex Pump head, Easy-load: FF-07518-12 ($190)
Mounting hardware for additional pump heads: FF-07013-05 ($22)

In order to avoid having to buy 2 peristaltic pumps, you can use the large pump for both. In order to do this, thread the size 15 tubing through size 73 tubing where it goes into the pump head.

Pressure Gauges

The pressure gauges and adaptors are purchased from Cole Parmer (1-800-323-4340)

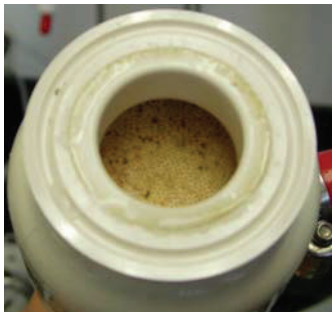
Pressure Gauge and adaptor to connect it:
0-30 PSI Stainless steel gauges: P-68007-03 ($62)
Gauge guards U-07359-08 ($118)
¼ female-female teflon adaptor: P-06376-31 ($15)
Kynar T-adaptors: P-30604-65 ($30 for a pack of 10)

These gauges need to be filled with mineral oil (we use catalog # U07359-50 from Cole Parmer). The gauge guard prevents the oil from getting into the rest of the system.

In the TFF set-up, you need a pressure gauge between the peristaltic pump and the TFF. Always keep the pressure below 10 PSI to avoid blowing up the viruses.

Hose Adaptor Kit

Used to attach tubing to large filters



The large filters require a hose-adaptor kit in order to connect the tubing (KA12-4PS-NY; $179).

With the medium filters, the tubing attaches directly to the filter.

Applying Pressure

It is necessary to apply pressure to the retentate tubing in order to force water through the pores of the filter (into the filtrate). Remember to keep the overall pressure below 10 psi.

When you have finished concentrating your sample, release the pressure clamp and allow the sample to wash over the filter for several minutes.

Taking it to the Field

To power everything in the field, we use the Honda EU1000i – available at motorcycle shops.

The peristaltic pump can be plugged directly into this generator

We have used this setup in a variety of environments – the only place that may cause problems from the generator is high altitude.

Wash Solution

Add 50 g NaOH to 5 liters of water

Mix until NaOH is in solution

Add 1 ml bleach and mix

Starting a new filter:

When you start a new filter, it needs to be rinsed of the storage preservatives.

1)Run DI water through the filter for 30 minutes. Make sure the pressure is adjusted to ~5 PSI

2)Run wash solution through the filter for 1 hour (for a large filter, you will need a lot of wash solution)

3)Rinse filters with DI water until both the filtrate and retentate fractions have a neutral pH

Cleaning the filters:

Filters should be cleaned with wash solution before and after processing a sample. Run at least 2 liters of wash solution through the medium filteres, and 5-10 liters through the large filters. Then run DI water through the filters (several liters) until the pH is neutral in both the filtrate and retentate fractions.

Storing the filters:

Store the filters at room temperature, wet with DI water. Do not store filters in wash solution. Make sure all the attachments points are plugged with the red covers, or that the ends of the tubing are in DI water. It is best if you do not allow the filters to dry out.

Other Recommendations

• It is important to look at your filtrate and retentate each time you process a sample, to make sure that everything is working. You should also perform counts on your input sample so you can determine recovery rates.

• If you are filtering a "dirty" sample with lots of particles or other things that would clog the filter, we recommend first putting the sample through Nitex mesh (pore size ~100 μm).

• Make sure to NEVER expose your filters to organic solvents such as chloroform. These will ruin the filters.

You should always monitor the filtrate and retentate to ensure that the filters are working properly. We fix our samples with 2% paraformaldehyde (Electron Microscopy Services), filter onto a 0.02 μm Anodisc (Whatman), stain with SYBR Gold (Molecular Probes), and then count bacteria and viruses using epifluorescence microscopy.

Nitex mesh is from Coastal supplies (619-562-8880) – we use cat #3-123-70 (pore size ~136 μm)

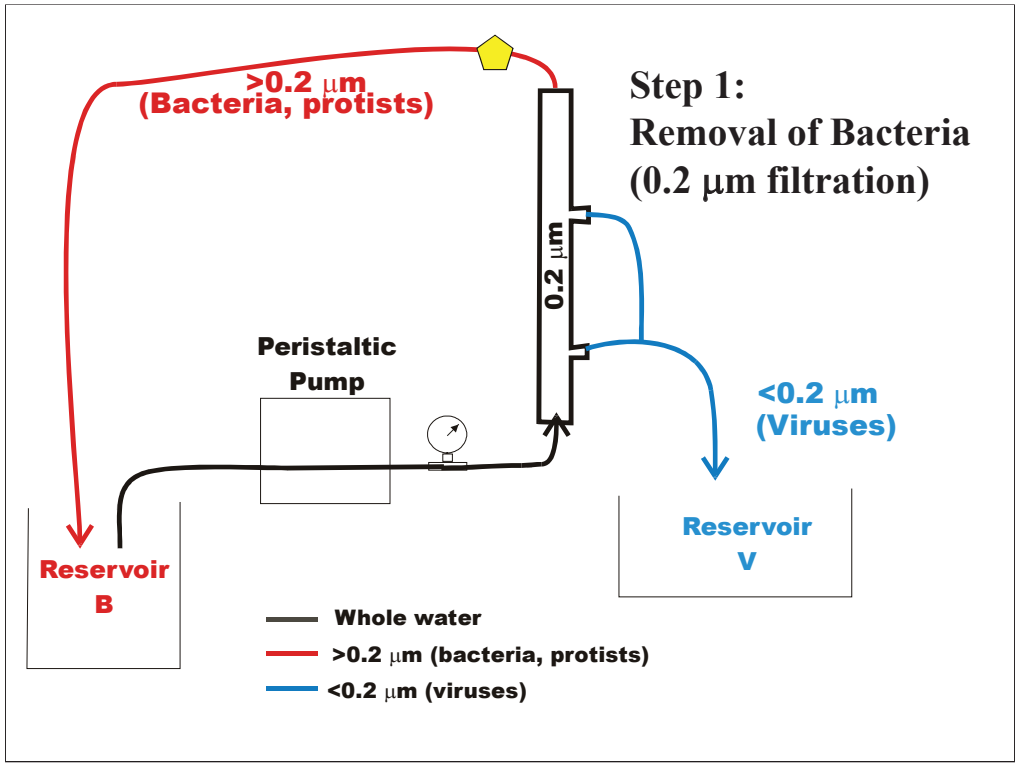
Other random parts you might want:

• various tubing connectors

• cable ties

• spare fuses for the peristaltic pump

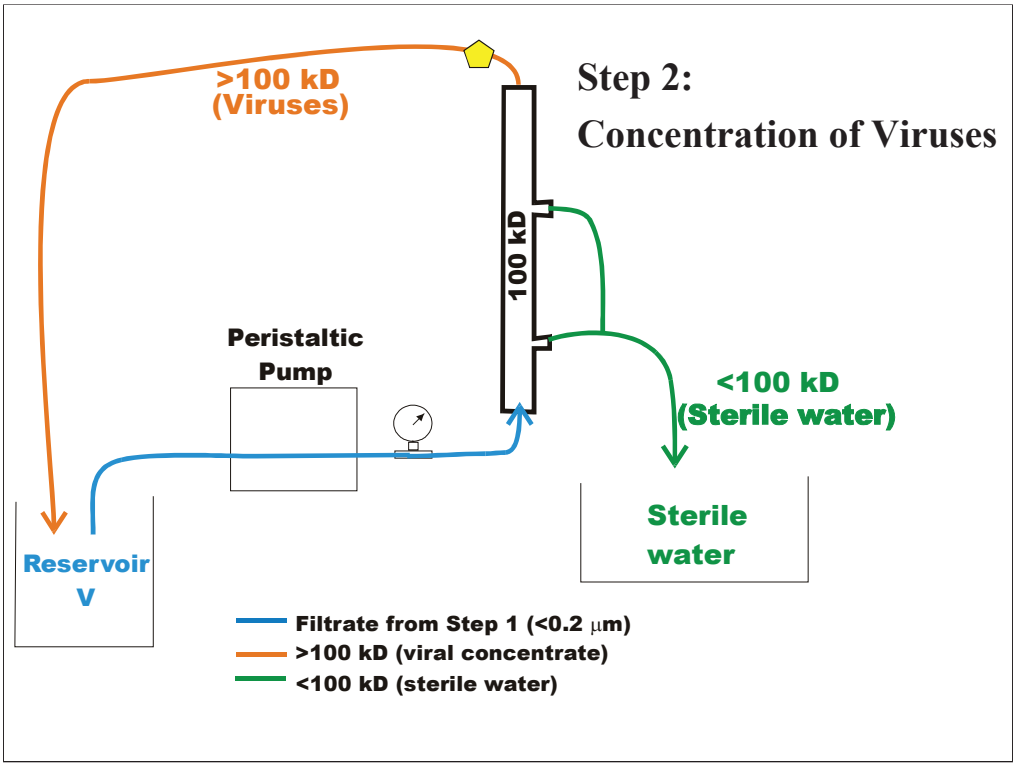
• Teflon tape



In Step 1, the retentate (red) contains Bacteria, which are larger than the pore size of 0.2 μm. This is recycled back into reservoir "B" until most of the volume has been 0.2 μm filtered. The small amount remaining in reservoir "B" is the bacterial concentrate. The filtrate (blue) contains the viruses (which are smaller than the 0.2 μm pore size), and is collected in reservoir "V" for future concentration (see Step 2 – next slide)

In both Steps 1 and 2, pressure should be applied on the retentate tube (shown by the yellow pentagon). This will force water through the filter into the filtrate.

It is important to keep the overall pressure of the sample below 10 psi. Pressures above this can compromise the viral particles. The pressure is regulated by 1) the speed of filtration (pump speed) and 2) the amount of pressure applied to the retentate tube.

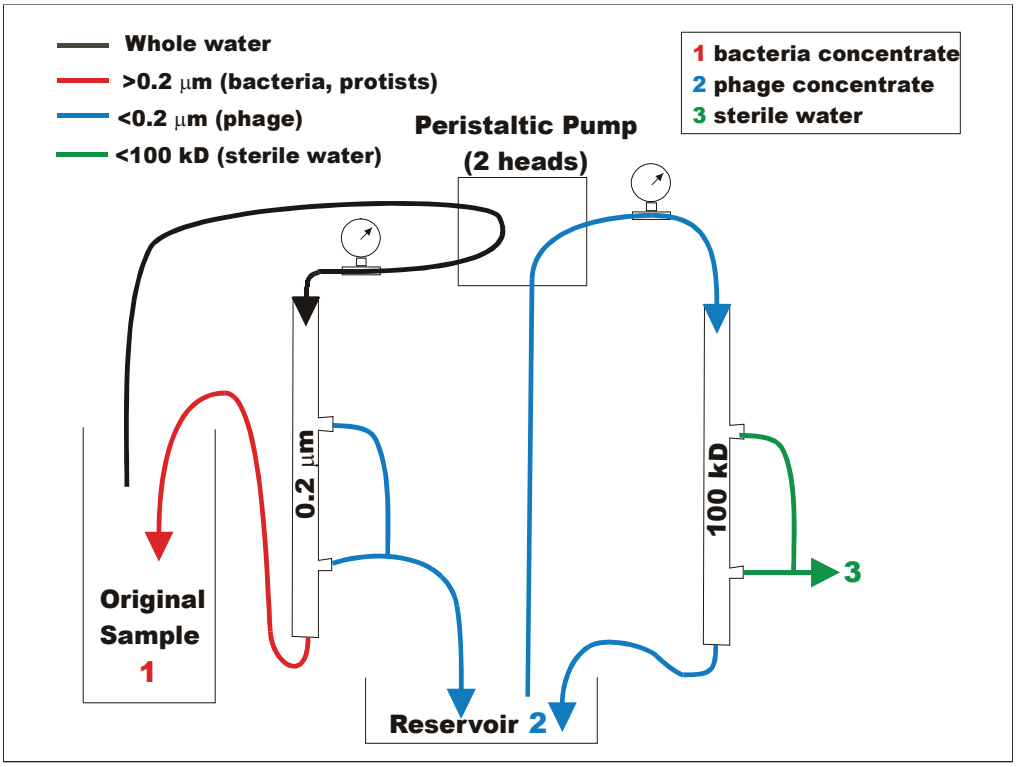


The filtrate from Step 1 (containing the viruses) is then concentrated in Step 2 using a 100 kD tangential flow filter. Since viruses are larger than 100 kD, they are in the retentate. The 100 kD filtrate is sterile water (with no microbes or viruses present).

If this set-up is run without any added pressure, essentially no water will come out the filtrate. To enable concentration of the sample, it is necessary to apply pressure on the retentate tube (shown by yellow pentagon).

The total amount of volume left at the end of the concentration process is determined by the void volume of the system (both of the filter and the tubing).

To save time with samples that are relatively clean (not many particles or debris), you can skip the 0.2 μm filtration of Step 1 and concentrate the whole water sample directly on the 100 kD filter. The concentrated sample can then be filtered through an impact filter to remove the bacteria.



Using a peristaltic pump with 2 heads, Steps 1 and 2 can be combined into 1 process.