

# **Installation Manual HTSL, Leap Autosampler, and CapNMR for One-Minute NMR**

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**Stepwise Instructions for HTSL/Pal/CapNMR  
Hardware Installation for One-Minute NMR  
Configuration: FSC-HTSL, FEP-Leap; FSC-CapNMR**

The HTSL is already plumbed in advance with the appropriate tubing. Note FSC = fused silica capillary.

The HTSL capillary connecting Valve 1 (V1) to Valve 2 (V2) should be disconnected from V2. You will find that this particular tube is connected to Port 2 (P2) in each valve. Use the yellow-handled hex tool to loosen the fittings on HTSL V2. Pull the capillary straight out of the fitting. Recut the tip with the special, black, diamond-blade capillary cutting tool. Check the tip under the magnifying glass to make sure it is a clean cut with no cracks or debris.

Test the HTSL to make sure it is primed with the solvent of interest. Fluid should come out of V1P2 quickly after a Run command is made. If not, reprime with the solvent of interest.

The connection at V2P2 will be connected to the Leap valve. Each Leap valve port should be squirted with acetone and dried with gas prior to any connections.

The HTSL capillary from V2P2 will be connected to the Leap valve. F-287 black nut (includes ¼" hexagonal flat), F-192 black ferrule, and F-242 green sleeve. First, using a piece of tape, anchor the capillary at the back of the green sleeve so its tip cannot slide past the end of the sleeve that goes into the port. Rinse the fittings with acetone to make sure they are clean. Squirt off the acetone (which gives an NMR background signal) with the canned gas just before assembly into the port. Once the nut is finger-tight, tighten it snug with a ¼" wrench.

All FEP tubing is cut with a clean razor blade. Make a square, 90 degree cut on a hard surface. The tubing should readily snap in two. Store the razor separately, keep it clean, and use it exclusively for cutting FEP. All tubing in baggies is already nicely cut. The FEP tubing on the spool should be given clean cuts.

The loop will be FEP tubing mounted between LVP3 and LVP6. The loop itself will be pre-cut and labeled in a plastic baggie. Use the tan F-334N nut (includes ¼" hex flat) and F-142N translucent ferrule to make the connection to the Leap valve. Again, squirt with water and dry off the tubing and fittings before insertion into the port itself. Once the nut is finger-tight, tighten it some more with a ¼" wrench.

Connect a section of FEP tubing (blue, 230-µm i.d.) to LVP3 with a F-334N nut, ferrule, and green sleeve. Cut the section to a suitable length and insert the other end in the provided waste container.

Connect about a 1-meter section of FEP tubing (clear, 75-µm i.d.) to LVP5. Use again, a F-334N nut, ferrule, and green sleeve. This section will go to a union, then a filter, then to the probe in the following manner:

Leap Valve Port 5----Clear FEP----P-779-01 Union----FSC----M-135 filter----FSC----Probe

The transition from FEP to the capillary on the probe occurs at the P-779-01 union. The FEP side uses a F-247 green sleeve (relatively large i.d.). The capillary side uses a F-242 green sleeve (relatively small i.d.). Both sides of the union use a tan F-331N nut and a translucent F-142N ferrule.

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Notes:

Glushka probe volume is 5 µL

Glushka typical sample pick-up volume will initially be 10 µL. OK to go down to 8 µL.

Loop volume will be 8 µL of black FEP with a 100-µm i.d.

## Parts and Tools in Order of Use

**Note:** FSC = Fused silica capillary

### HTSL

- To prime, the nut on the pump face may need to be loosened with a ¼” metal wrench
- Yellow-handled long hex wrench (3/32”)
  - To loosen valve nuts on HTSL Valve 2 only
- M-135 filter
  - Replace capsule with new tan capsule
  - Check capillary tips carefully before reassembly; re-cut if necessary

### Leap Valve

- F-334N tan nut + F-142N translucent ferrule used in Leap valve ports 2, 3, 5, and 6 for FEP connections
- F-287 black nut + F-192 black ferrule used in Leap valve port 4 only for FSC connection from HTSL
- Both the tan F-334N and black F-287 nuts can be tightened snug with a ¼” metal wrench.
- F-247 green sleeves (large hole; for FEP)
- F-242 green sleeve (small hole; for FSC)
- FEP tubing in pre-packaged segments (just squirt clean with acetone and install)
  - Clear (75 µm i.d.)
  - Black (100 µm i.d.)
  - Blue (230 µm i.d.)

### Leap Valve-to-Probe Flowpath

- P-779-01 union
- F-331N tan nut + F-142N translucent ferrule
- F-247 green sleeves (large hole; for FEP)
- F-242 green sleeve (small hole; for FSC)
- M-135 filter with tan capsule + F-185 tiny green sleeves for FSC into this filter only

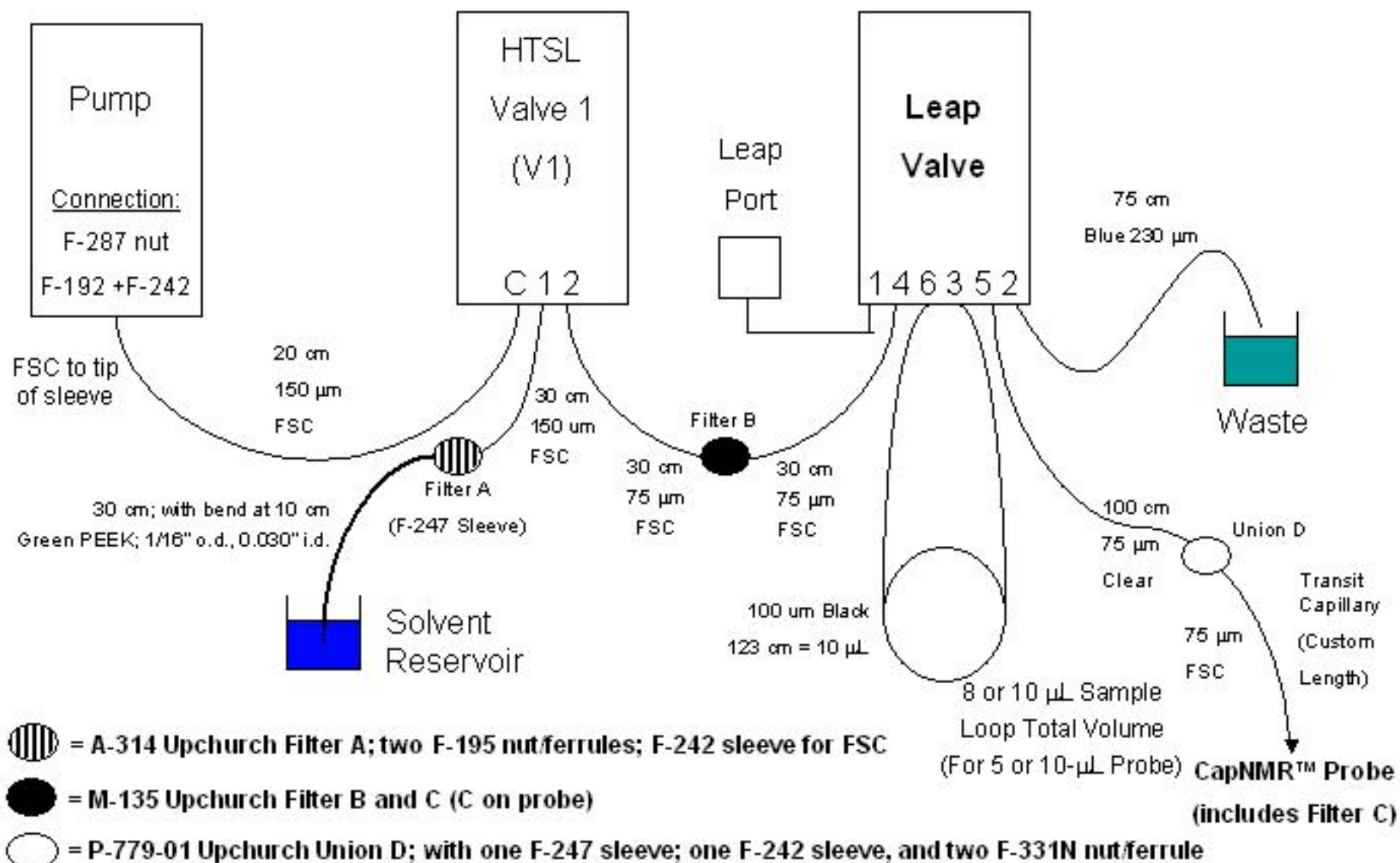
### Priming the HTSL

- Use parts in labeled baggie (nuts, tubing, adapter, bottle with hole in cap)
- Syringe is a 1-mL (1000 µL) Hamilton gas-tight
- Use deuterated solvent
- Use tightening device (P-298; black plastic) to loosen and re-tighten the plug in the pump side port

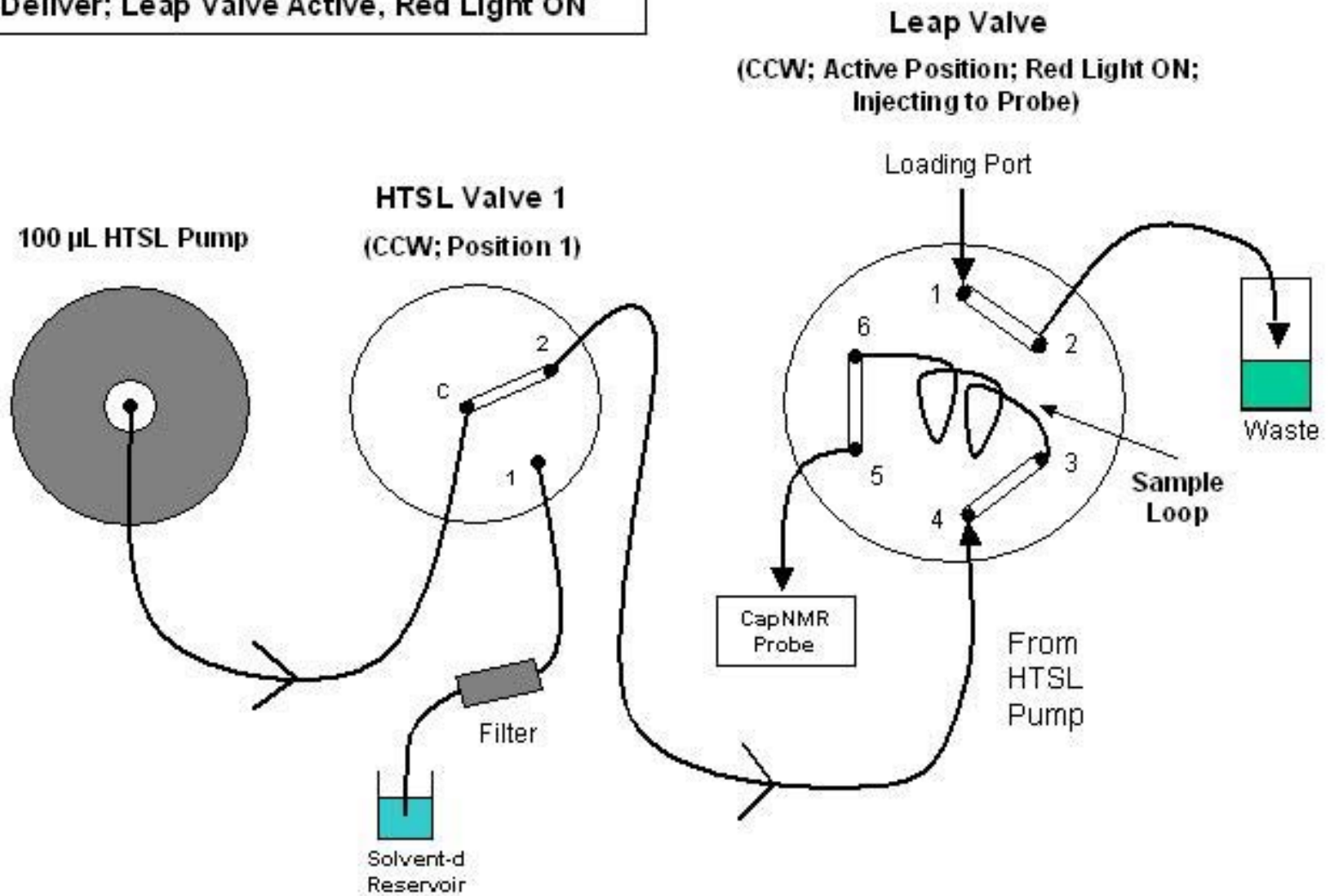
# HTSL Tubing Layout

## FSC-HTSL and FEP-Leap Autosampler; FSC Probe

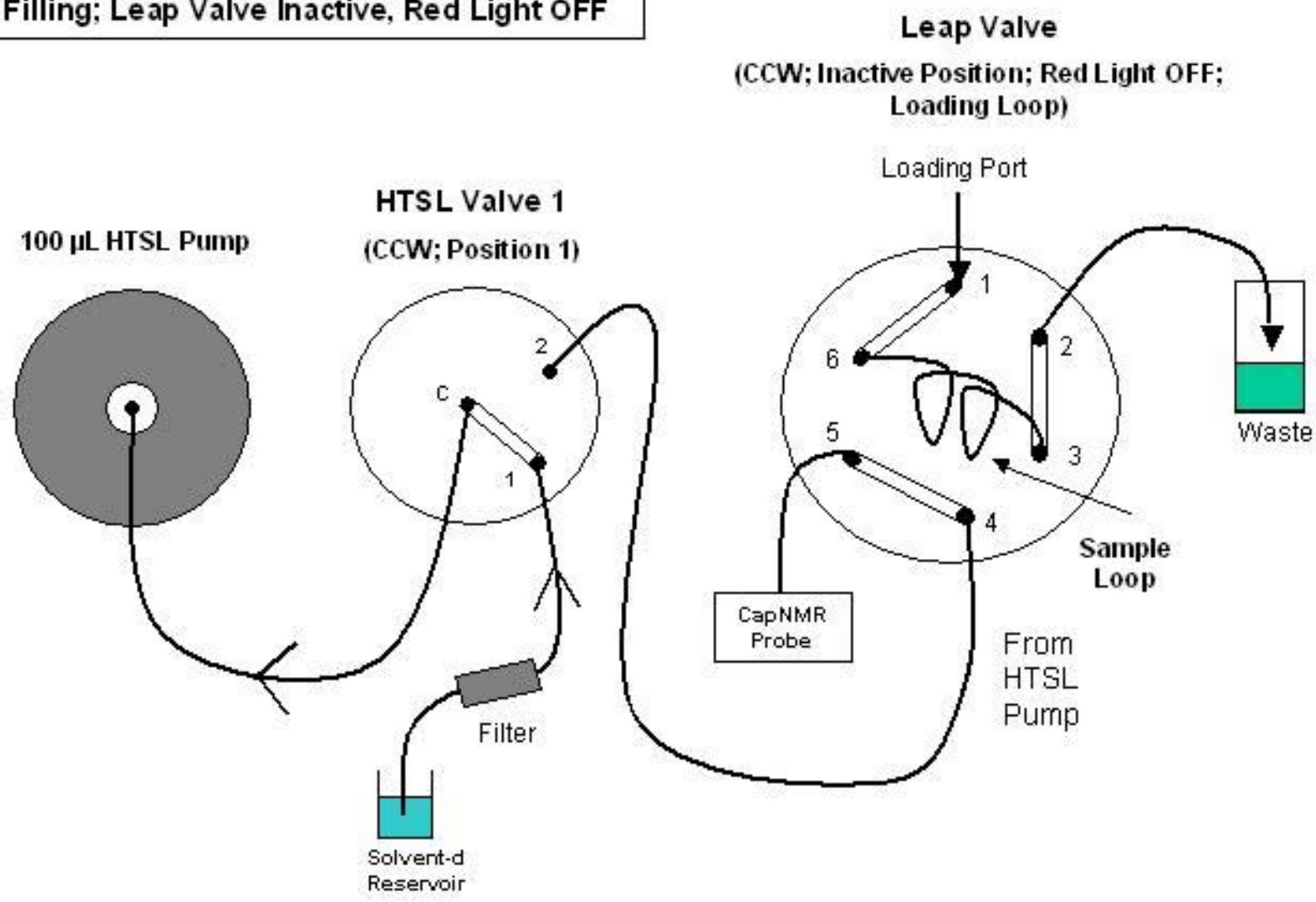
FEP tubing; 1/32" o.d.; i.d. as shown



**HTSL Valve Diagram for FSC-HTSL and FEP-Leap:  
HTSL Deliver; Leap Valve Active, Red Light ON**



**HTSL Valve Diagram for FSC-HTSL and FEP-Leap:  
HTSL Filling; Leap Valve Inactive, Red Light OFF**





## Calibration Procedure for Delivery Volume on HTSL/Leap System

### Preparing the HTSL

- Prime the HTSL if necessary
- With the flow path completely assembled from HTSL, to Leap Valve, to transit tube (not probe yet), put the Leap valve in the Red Light ON position using the gameboy. This is the Active position, but the gameboy will indicate Inactive because that's what it *will* do if you hit the button.
- Using Protyle, set the HTSL to Fill and Deliver at 15  $\mu\text{L}/\text{min}$ , and to a Delivery Volume of 90  $\mu\text{L}$ . Trigger a run and check for leaks along the entire flow path and make sure fluid comes out of the tube going to the probe
- Put the Leap Valve in the Red Light OFF position, and trigger another run to rinse the valve
- Reset the Leap valve to the Red Light ON position
- Connect the probe, and trigger another run again checking for leaks, and be sure fluid comes out of the probe exit tube. The exit tube need not have an extension capillary segment attached to it.
- Once rinsing is complete, set the HTSL Delivery Volume to 60  $\mu\text{L}$ .

### Preparing the NMR

- Take a one-scan proton spectrum with the CapNMR probe from about 0-10 ppm using a reasonable point density. Set the acquisition time to 4 sec, and d1 to zero. If spectra are acquired successively on flow, each spectrum corresponds to 1  $\mu\text{L}$ .
- On a Varian system, with NT = 1, array NT to 60 elements, with no increment between array elements. Check that time = 4 min 0 sec for the entire array.

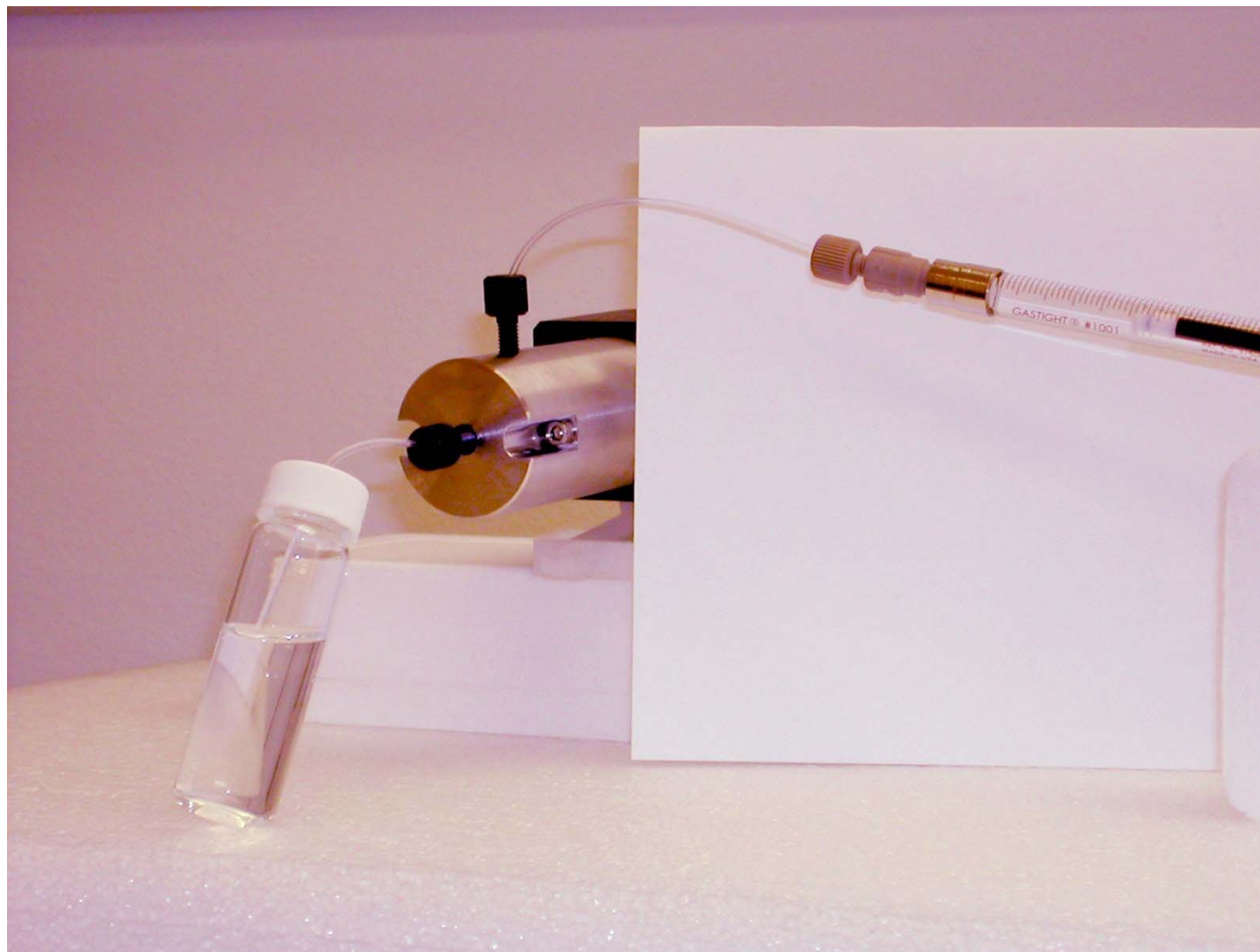
### Preparing the Leap Autosampler

- Using the Leap gameboy, put the valve in the Red Light OFF state
- Using a 25- $\mu\text{L}$  syringe mounted with a 22-gauge needle, fill the syringe with sample, and insert into the Leap port screwed into port 1 of the Leap valve.
- Load the loop manually via the port using the syringe and confirm that fluid exits the blue waste tube.

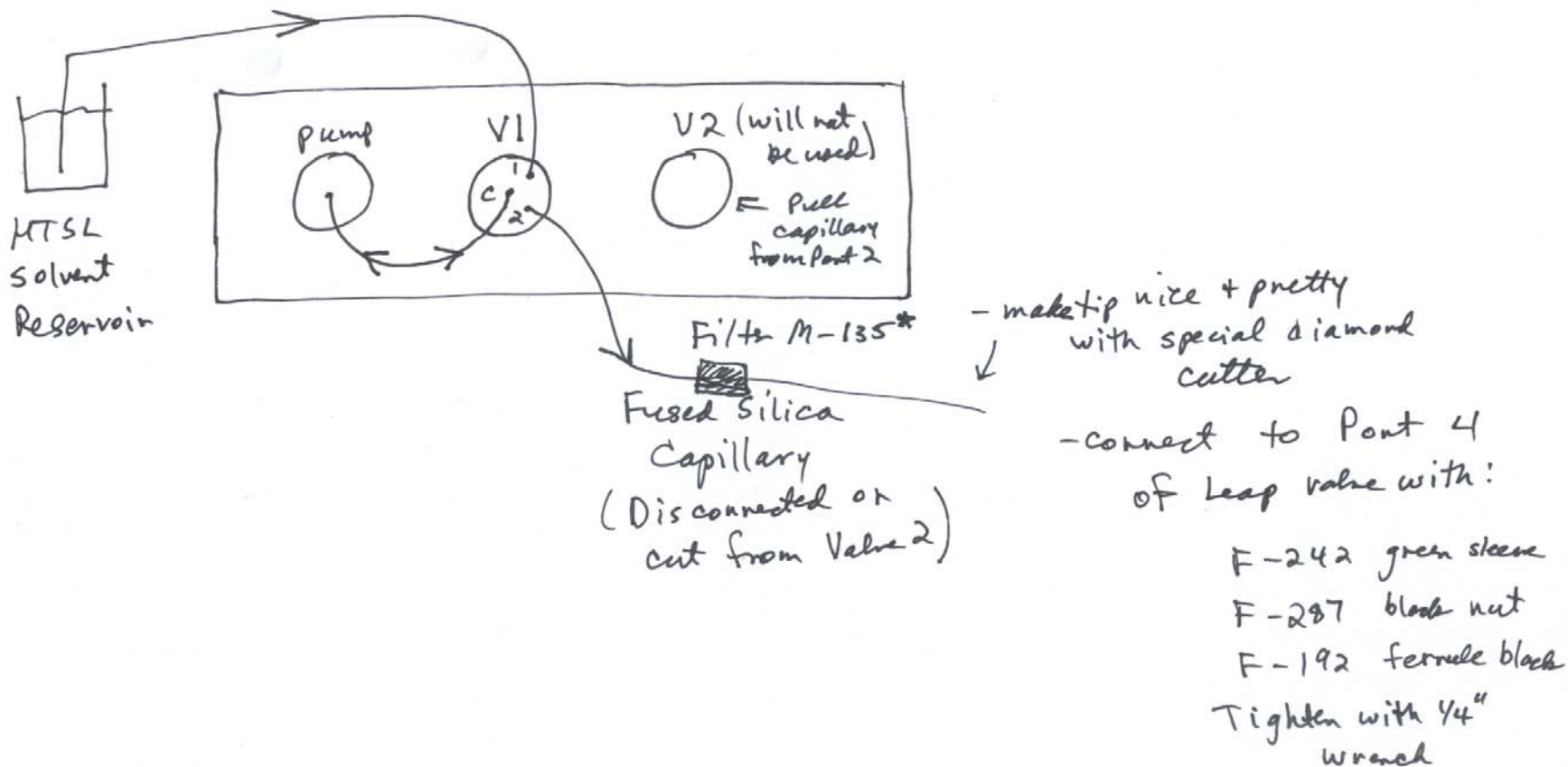
### Triggering the Calibration Run

- Put the Leap valve in the Red Light ON position, then simultaneously:
  - Trigger the HTSL
  - Type ga <return> on the NMR workstation.
- Use the dssh command on the NMR to display stacked spectra horizontally; update frequently
- You will see the flow profile appear according to the loop volume used in the Leap valve
- The maximum should be repeatable +/- one spectrum; do several repeats. That maximum spectrum is also the number of  $\mu\text{L}$  to set the HTSL Delivery Volume to using Protyle. This is the calibrated Delivery Volume for the HTSL

## Priming Configuration



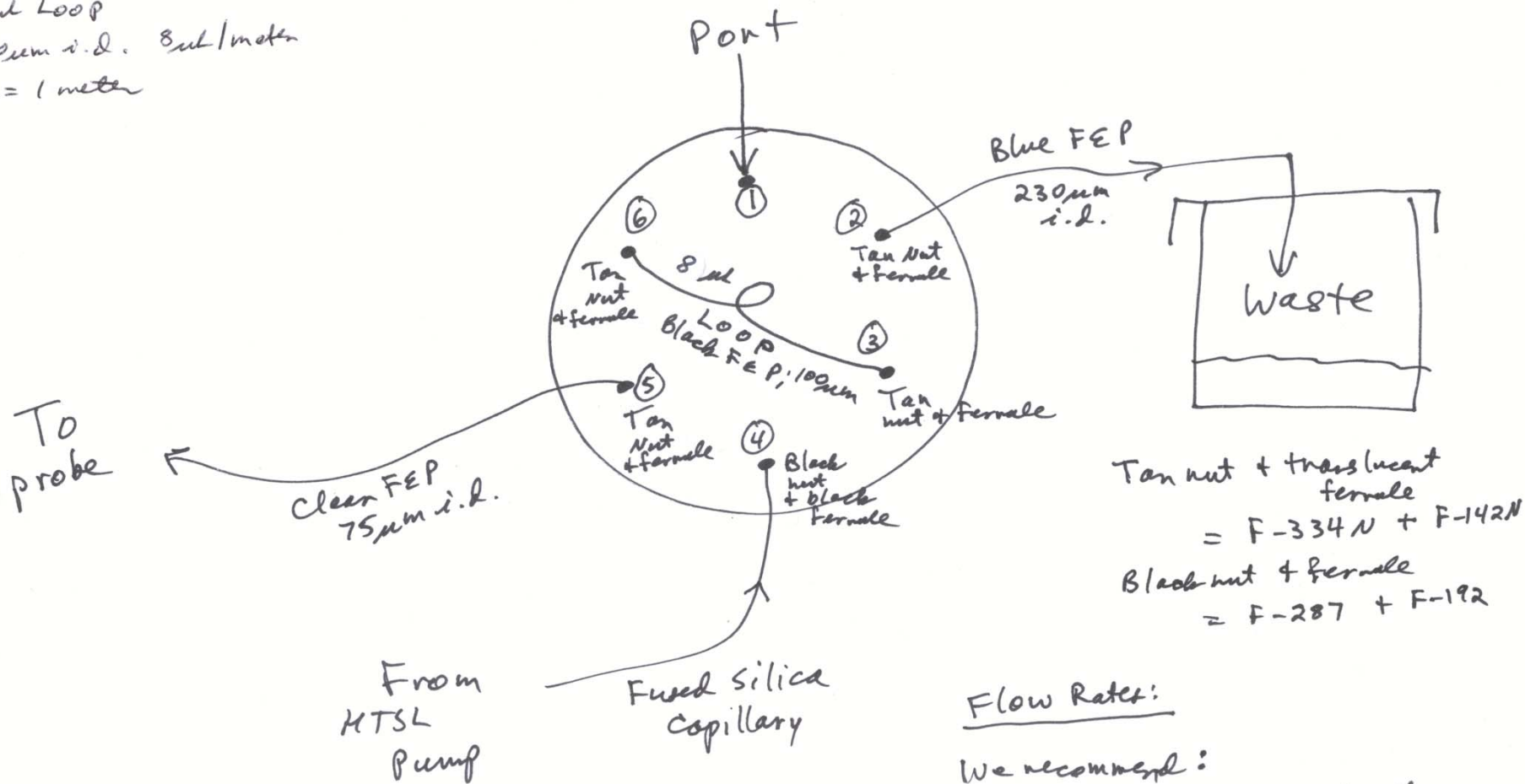
# MTSL-to-Leap Valve Flowpath



\* Be sure to use an M-135 filter here even if an old model needs to be replaced  
Also, needs F-185 tiny green sleeves

# Leap Valve Connections

8ul Loop  
 100um i.d. 8ul/meter  
 L = 1 meter



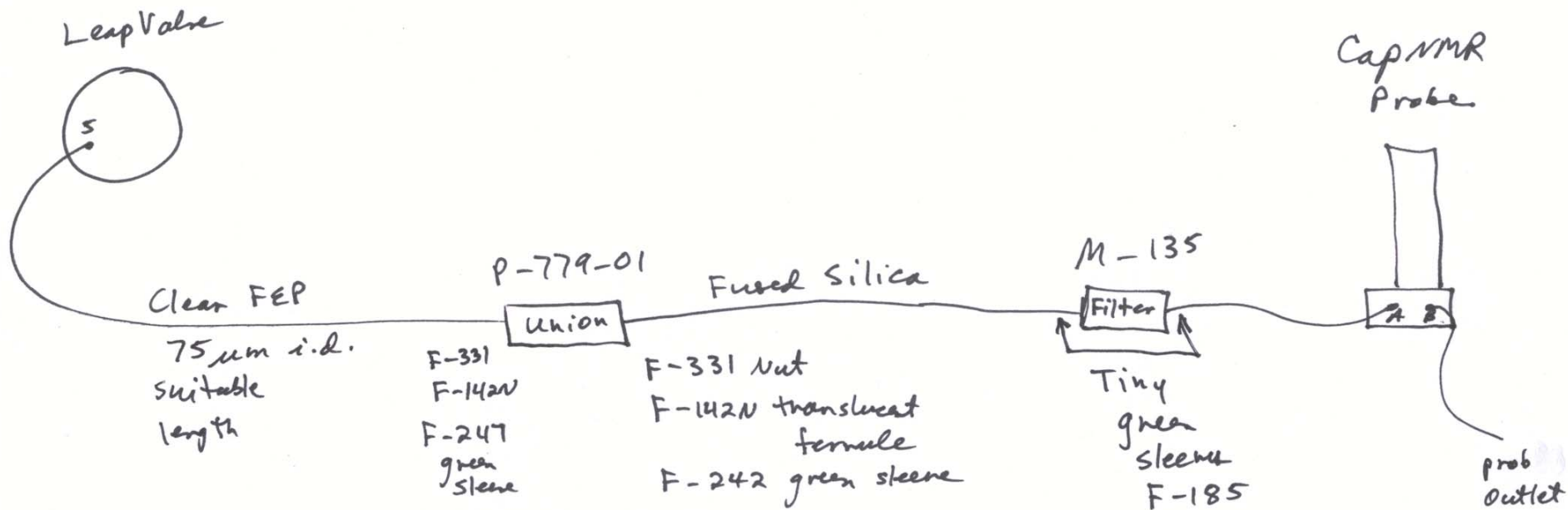
## Flow Rates:

We recommend:

- pick up the sample at 1ul/sec
- Load the sample into the loop at 1ul/sec
- Set HTSL to Deliver at 15ul/min + Fill at 15ul/min

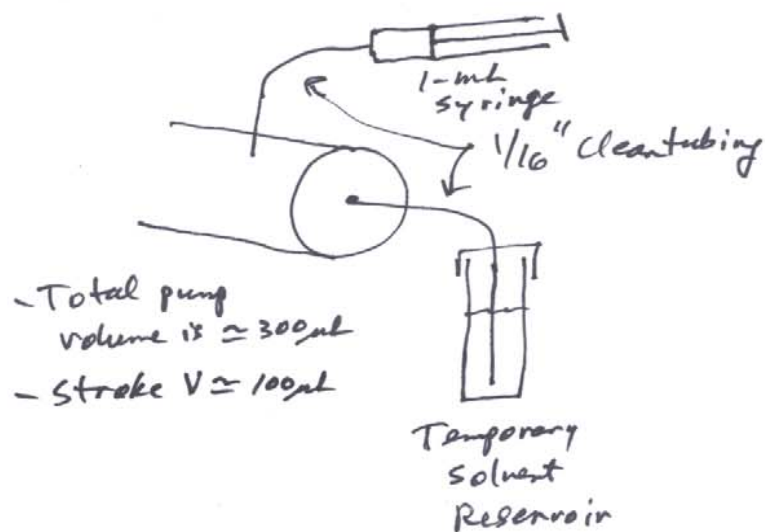
Note: John has a 5- $\mu$ l probe with 50um i.d. tubing in the form of FSC (Fused silica Capillary)

# Leap Valve - to - Probe Flow Path



## HTSL Priming Configuration and Procedure

① With Pump in Empty position (use Protyle):



- Remove solvent uptake flow path from front of pump (capillary, nut, filter, grommet PEEK tubing)
- Begin with empty syringe and the desired deuterated solvent in the temporary reservoir
- Cycle solvent back and forth until bubbles are expelled, or appear only at the top of the syringe
- Fill the syringe and move to step 2

②

- Remove tube above from front of pump
- push out a little fluid
- Re-connect original solvent uptake flow path to front of pump
- Push fluid out of flowpath rinsing it well + dispose of fluid
- put solvent uptake flowpath into permanent solvent reservoir
- pull syringe all the way back and wait 5 min. A cavitation bubble will form. Allow for fluidic equilibration. Raise Reservoir above Pump

- ③ Detach syringe only at the tip of syringe (unscrew syringe from adapter)
- ④ Detach tube at side port
- ⑤ Re-plug side port