One-Minute NMR
Fluidics Manual 2008

For HTSL,
Leap Autosampler, and CapNMR

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For HTSL, Leap CTC Autosampler, and CapNMR Probe

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For OneMinuteNMR software directions and suggestions:
http://protasis.com/OneMinuteNMR/OneMinuteNMRQuickStartGuide.pdf

Parts and Tools in Order of Use

HTSL

- To prime the HTSL pump after a manual solvent switch, the plug on top of the pump head will need to be removed (Upchurch makes a helpful nut extender tool, Part# P-298)
- Silver specialty wrench
  - To loosen red port nuts on HTSL valves (Upchurch# P-297)
- M-542 tan filter (Upchurch)
  - Replace capsule with new tan capsule (capsule alone is M-132; ferrule is F-112)
  - Check capillary/tubing tips carefully before reassembly; re-cut if necessary
- CUCKF white filter (Valco)
  - Replace filter disks with new disks (2 um porosity part # CFE-S2)
  - Check capillary/tubing tips carefully before reassembly; re-cut if necessary

Leap Valve

- F-334N tan nut + F-142N translucent ferrule used in Leap valve ports for FEP tubing connections
- The tan F-334N nuts can be tightened snug with a ¼" metal wrench to ensure a good seal.
- 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.) for transit lines - 4.6 uL/meter
  - Black (100 μm i.d.) for sample loop - 8.1 uL/meter

Leap Valve-to-Probe Flowpath

- P-779-01 union (tan)
- F-334N tan nut + F-142N translucent ferrule
- F-247 green sleeves (for SS and FEP)
- M-542 filter with tan capsule included (separate replacement is M-132; ferrule is F-112)
- CUCKF (Valco) filter with white union body and fittings (Replacement filter disks CFE-S2)

Priming the HTSL

- Use parts in labeled bag (nuts, tubing, adapter, bottle with hole in cap)
- Syringe is a 1-mL (1000 μL) Hamilton gas-tight (For Discovery Towers, syringe not required)
- Use deuterated solvent
- Use tightening device (P-298; black plastic) to loosen and re-tighten the plug in the pump side port
- Prime with pump in empty position; use Protyle
- Priming should be quite easy

Well plates and Sample Containers

- We recommend MicroLiter Analytical as the supplier for well plates and vials. (1-888-232-7840).
- For people who prefer not to use well plates (where the sample actually rests in the well):
  - Inserts are part #11-0000-100 (100-pack); $71.75 in April 2005
  - Vials are part #11-5200 (100-pack); $15.50 in April 2005
  - Caps are Teflon lined, part #11-0055N (natural in color; other colors available); $15.50 in April 2005

Leap Operational Notes

Changing Well Plates & Tray Declarations

- Menu (F1)
  - Setup (F3) <enter>
    - Objects <enter>
      - Trays <enter>
        - Select Desired Tray (usually Tray01, Tray02, Tray….) <enter>

- Scroll down to highlight 'Tray Type' <enter> and scroll through options
  - VT54 – Vial Tray with 54 locations (blue)
  - MT96 – Microplate Tray with 96 wells (short)
  - DW96 – Deep Well tray with 96 wells (tall)

- Confirm/Adjust XYZ to coincide with Tray position 1 and well 1 (see detailed directions below)
  - Be sure to save each coordinate
  - It may go around to the four corners of the Tray

Wash the Leap Syringe (Clean the needle or Remove a bubble)

- Menu (F1)
  - Clean Syr (F2) <enter>
    - Select a Wash Station <enter>
      - Enter the number of times the syringe should rinse <enter>

This option will move the leap syringe to the desired wash station and will rinse the full length of the syringe body. For new, or completely dry syringes, it is recommended to slightly pre-wet the syringe with solvent to displace some of the air.

Setting Needle Penetration Depth for a Particular Tray

- *Setting the Tray Holder XYZ position (performed at installation & described elsewhere) also enables the Needle Guide to find corner positions of a specified Tray (or well plate)*
- This procedure guides a user to select a Tray, then adjust the needle penetration for a vial resting in that Tray. All of the XYZ positions for the Tray Holder, Wash Stations, Injector Port, and Waste should be calibrated prior to this procedure

- Menu (F1)
  - Utilities <enter>
    - Tray (select Tray – Tray## or Stack - Stk1-##) <enter>
      - Needle Penetr <enter>

  - Start by entering a Needle Penetr depth of 28.0 mm <enter>
    - Movto 001 (F3), Needle Penetr <enter>, Dial depth downward till downward syringe motion halts, back up 0.1 mm, <enter>
    - Movto 009 (F3), Needle Penetr <enter>, Dial depth downward till downward syringe motion halts, back up 0.1 mm, <enter>

        - Movto 054 (F3), Needle Penetr <enter>, Dial depth downward till downward syringe motion halts, back up 0.1 mm, <enter>
        - Other Tray Types will have different numbers for the corner positions
    - Home

Leap Syringe Replacement

- Prime the replacement syringe manually. Use fresh (dry) solvent, and remove all bubbles.
- ***On the gameboy, hit Menu (F1), Chang Syr (F1) and wait for the Z-arm and syringe to position itself***
- On the autosampler syringe arm, slide up the clear plastic cover.
- Near the top of the syringe, loosen the red knob by turning it to the right.
- At the midpoint of the syringe, rotate the small, black arm by 90 degrees to restrain the syringe body.
- Pinch together the pair of black needle guides that the needle slides through in normal use.
- With the needle guides pinched together, remove the syringe by gently tipping it forward and up.
- Put into place the new syringe, again pinching together the black needle guides.
- Be sure the syringe is against the mounting plate at its top and bottom.
- Rotate the small, black arm back to its original position.
- Tighten the red knob against the syringe plunger by turning the knob to the left.
- Slide closed the clear plastic cover.
- On the Leap Gameboy, hit the ESC key (repeatedly if needed) to return to the top of the menu.
- One at the top of the menu, hit F1 twice.
- Disable the syringe detection feature by following the on-screen directions.
- Enter the correct volume for the syringe presently in place.

Note:

- We presently recommend a 50-uL syringe (Leap part #LMK.2620617-2P is the two-pack; approx. $122.00 as of 2005). Syringes of 25 μL and 10 μL are available, but might cause carryover problems due to inadequate rinsing.
- The One-Minute NMR software is designed to accommodate a new syringe volume by following the above procedure. The software reads the declared volume from the autosampler when the RunProcess is started, and responds accordingly. However, the syringe volume cannot be less than the sample pick-up volume.
- The autosampler does not need a matched mounting plate to accommodate a different syringe volume. Simply turn off the Syringe Detect feature, as described elsewhere.

Rinsing the Wash Station and Tubing with Solvent after refilling Solvent Reservoir

- To replenish the Wash solvent, close the stopcock at the base of the Wash bottle
- Disconnect the tube at the black nut that goes into the white stopcock at the base of the bottle
- Rinse the bottle with fresh solvent and drain some solvent out through the stopcock
- Place the Wash bottle back into position, re-connect its tube, and open the stopcock
    - Menu (F1)
        - Utilities <enter>
            - Wash Station <enter>
                - Select desired wash station <enter>
- ***Press Act Valve (F2) to open the valve (located in the solenoid under the Wash Station)***
- Rinse the tube until all the bubbles in it are flushed out (several seconds)
- Press Deact Valve (F2) to close the solenoid valve

- When F2 reads Act Valve, the solenoid is closed
- When F2 reads Deact Valve, the solenoid is open

- Home

Accessing the F3 Key

- For administrative privileges; Usually used to define something for the first time
- Not used to select something already defined
- The sequence is:
  - Menu (F1)
    - Setup (F3) <enter>
      - Objects <enter>
        - Syringes (for instance) <enter>
          - Home

Throwing the Leap Autosampler Valve

- Menu
  - Utilities <enter>
    - Injector <enter> (hit <enter> again to select default injector)

Pressing Act Valve (F2) puts the valve in the red light Off position = injection port connected to loop
Pressing Deact Valve (F2) puts the valve in the red light On position = injection port not connected to loop

- Home

Mounting the Syringe

- Menu (F1)
  - Chang Syr (F1)
    - Mount the syringe in the blue bracket and hit Continue when finished
    - Ensure the correct syringe is highlighted <enter>
      - Home

Filter Mounting and Needle Penetration Adjustment for Injector Port *(Optional)*

- Screw the OpTech filter into place in the valve following these steps:
  - It is important that this be done first
  - Be sure the metal post of the filter is seated in the very bottom of the valve
  - Place the plastic needle guide (part #PAL NdlSealP) on top of the filter itself while you tighten the black filter body; push the guide down onto the filter with your finger while you tighten
- Once the filter is in place in the valve, place the needle sleeve (part #PAL NdlSealP) into it
- Next, mount the metal Leap port in the filter, but slide the needle through the tube of the needle sleeve before tightening the metal port
- With the needle in place and bottomed out against the filter, tighten the Leap port until firmly snug
  - Check that you can repeatedly bottom out the needle against the filter by removing and replacing the needle several times
  - Proceed to adjust the needle penetration
- Menu, Setup, Objects, Injectors, LC V1v1, Z, <enter>, and set Z to 60 mm or less, <enter>
- Needle Penetr(ation), and set Needle Penetr to 5 mm or less, <enter>
- Hit Check Pos(ition) F1

- Choose Z, <enter>, and adjust it using the black needle guide as reference; save with <enter>
- Choose Needle Penetr(ation), <enter>, adjust to clunk, and back up 0.1 mm, <enter>
- Verify the Needle Penetr setting by
    - Select Needle Penetr, <enter>, and listen for thunk; it might thunk a little, but not as badly. If it thunks badly, readjust the Needle Penetr till it thunks back up an additional 0.1 mm, and hit <enter>.
    - Repeat this step as needed. It often takes several adjustments, but is then consistent.
    - When adjusted, hit Movto Zero
- Home

Tray Holder Declarations

- A well plate is called a Tray; the Tray is placed on the Tray Holder
    - The default Tray Holder is called THldr1
    - The default Stack is called Stk1-01

    - Menu (F1)
        - Setup (F3) <enter>
            - Objects <enter>
                - Tray Holders <enter>
                    - Insert new Tray Holder

        - Proceed to create the name THldr1 or THldr##
        - Proceed to dial in the xyz coordinates for it
        - Save them and check the position
            - Home

Syringe Definitions – General Specifications

- Access the Syringe definition menu via the F3 key using this procedure:
    - Menu (F1)
        - Setup (F3) <enter>
            - Objects <enter>
                - Syringes <enter>

- Enter the settings for the 50-µL syringe as defined below

Syringe Definitions – Volume and Name

- The syringe detector is usually left Off unless the sample return feature is being used. It is better to simply define the syringe in use as described elsewhere (see Syringe Detection On/Off)

- The 50-µL syringe is initially not defined, and has to be at installation on each Gameboy
    - Menu (F1)
        - Setup (F3) <enter>
            - Objects <enter>
                - Syringes <enter>
                    - Insert Syringe (F2)

- Dial in a syringe name by character, hitting <enter> after each character, then Enter (F4)

- Next, see the General Specifications sequence below, and enter the appropriate definitions. However, this is usually done for the user at Installation.

Syringe Detector On/Off

- Provides the i.d. of the syringe to the Actual Id entry above (if On)
    - Menu (F1)
        - Setup (F3) <enter>
            - System <enter>
                - Syr Detect <enter>

- Simply Scroll through On / Off <enter>
- The syringe detector provides no other automatic information
    - For those using sample return, this feature should be turned on
    - For those not using sample return, this feature should be turned off.

Default Syringe Settings in a New Pal (for 100 uL syringe)

Default settings for 100-µL syringe, as received from Leap are:

- Syringe Id = 4
- Actual Id = 0
- Min Volume = 10.0µl
- Max Volume = 100µl
- Scale length = 60.0mm
- Standby Pos = 0µm
- Min Speed = 1.0µl/s
- Max Speed = 200µl/s
- Fill Volume = 0nl
- Fill strokes = 0
- Overfill = 0%
- Pullup Del = 500ms
- Fill Speed = 50µl/s
- Eject Speed = 100µl/s
- Inject Speed = 100µl/s
- Motor Drive = MPlgMed
- Heater = None

Default Settings for a 50-µL Syringe (installed by Protasis/MRM)

- Syringe Id = 3 (not usually overwritten)
- Actual Id = 3 (not usually overwritten)
- Min Volume = 100nl (usually overwritten)
- Max Volume = 50µl (not usually overwritten)
- Scale length = 60.0mm (not overwritten)
- Standby Pos = 0µm (not overwritten)
- Min Speed = 100nl/s (can be overwritten)
- Max Speed = 50µl/s (can be overwritten)
- Fill Volume = 0nl (done in advance of pick-up; not usually done)
- Fill strokes = 0 (done in advance of pick-up; not usually done)
- Overfill = 0% (not usually overwritten)

- Pullup Del = 500ms (can be overwritten)
- Fill Speed = 25ul/s (usually overwritten)
- Eject Speed = 25ul/s (not usually overwritten)
- Inject Speed = 10ul/s (usually overwritten)
- Motor Drive = MPlgMed (don’t use High)
- Heater = None
- The 50-µL syringe is now set to these parameters
- Home

Setting the Syringe Motor Drive

Standard setting is MPlgMed (medium). In general, don't use the low and high settings, though the high setting might be used in the case of a polyethylene plunger tip only. For our standard, 50-µL, Teflon-tipped plunger, the high setting is troublesome, and drives the needle too hard.

- Menu (F1)
  - Setup (F3) <enter>
    - Objects <enter>
      - Syringes <enter>
        - Scroll to 50µL <enter>
  - Scroll to Motor Drive <enter>
  - Scroll to MPlgMed <enter>
    - Home

Setting XYZ Coordinates

- Five locations must be set:
  - Tray Holder (bottom of black needle guide even with to surface of hole)
    - Called CStack1
    - The Tray Holder holds the Tray (or well plate)
  - Wash1
  - Wash2
  - Injectors
    - Port labeled V1v1
    - Waste (also listed under Injectors)
- Setting Coordinates for Tray Holder, Wash Stations, Injector Port, and Waste
- Menu (F1)
  - Setup (F3) <enter>
    - Objects <enter>
      - Scroll down to select object, choose location and set xyz coordinates
  - Always finish the setting with <enter> to save the coordinate setting
  - Home

Updating your One Minute NMR Version

The Web page for One Minute NMR software updates is:

http://protasis.com/OneMinuteNMR/OneMinuteNMRSetup.zip

Upgrade Instructions

- Uninstall the previous version with Control Panel/Add or Remove Programs (Remove the old versions of both One Minute NMR Run Process and OneMinuteNMR)
- Re-start the computer
- Download and extract the zipped archive to a CD or directory on your hard drive (OK to write over the old, zipped version)
- Open OneMinuteNMRSetup.zip
- Install the new versions of:
    - One-Minute NMR/Website Setup/Setup.exe
    - One-Minute NMR/Run Process Setup/Setup.exe
        - In the Select Installation Folder, be sure to select "Everyone"
- Open the Run Process and select the Database/Update Database menu item

Here is a typical New Revision release notice e-mailed by Bob Albrecht:

From: Bob Albrecht [mailto:b.albrecht@protasis.com]
Sent: Friday, September 30, 2005 12:53 PM
To: You
Subject: One-Minute NMR 1.1.14 Limited Release

Dear One-Minute NMR Customers,

Protasis has released version 1.1.14 of One-Minute NMR. This release has a number of additions and fixes to address intermittent stoppages. Automated structure confirmation features are also included that can be activated at a later date.

To install this release you will need download * http://ht-nmr.com/OneMinuteNMR/OneMinuteNMRSetup.zip and extract the archive to a CD or directory on your hard drive. If you have not installed version 1.1.12 or 1.1.13 you will also need to install the new HTSL firmware to enable overpressure and error sensing. The firmware is located at http://protasis.com/OneMinuteNMR/HTSL2_68.zip

One-Minute NMR Installation Instructions

1. Uninstall the previous version with Control Panel/Add or Remove Programs
2. Re-start the computer.
3. Install the new versions: One-Minute NMR/Website Setup/Setup.exe and One-Minute NMR/Run Process Setup/Setup.exe, be sure to select "Everyone".
4. Open the Run Process and select the Database/Update Database menu item.
5. If you have a Protasis Solvent Conditioner: One-Minute NMR/Solvent Conditioner/Setup.exe, be sure to select "Everyone".

HTSL Firmware Installation
Contact Protasis support.

Please call if need help with the installation, especially if you are unsure about the Pumps section.

Best Regards,

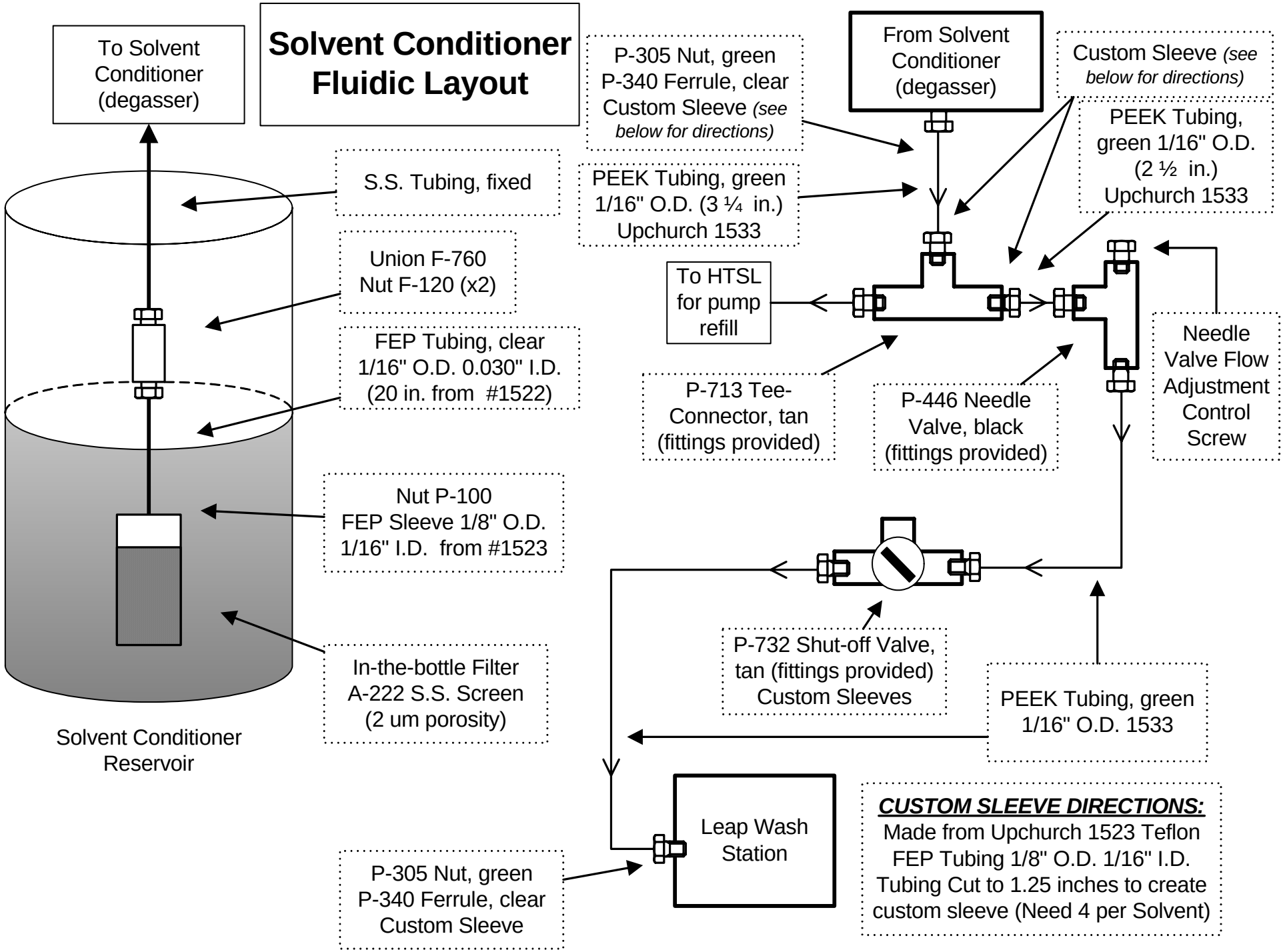
Bob Albrecht
Protasis Corporation

Tel: 434-823-6312
Cell: 434-249-1747

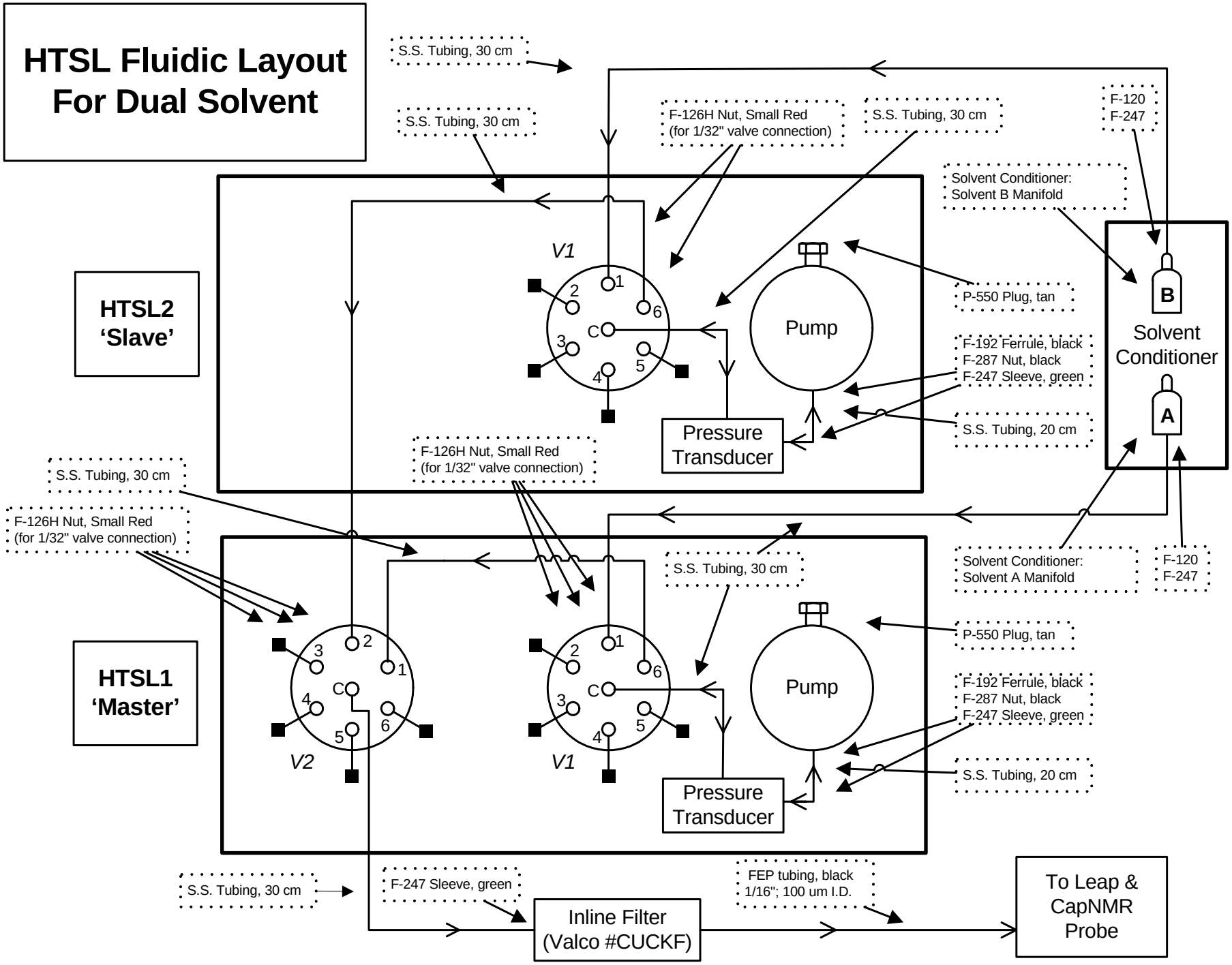
NOTE: It will be available at http://protasis.com/OneMinuteNMR/OneMinuteNMRSetup.zip
by 2:00PM EST

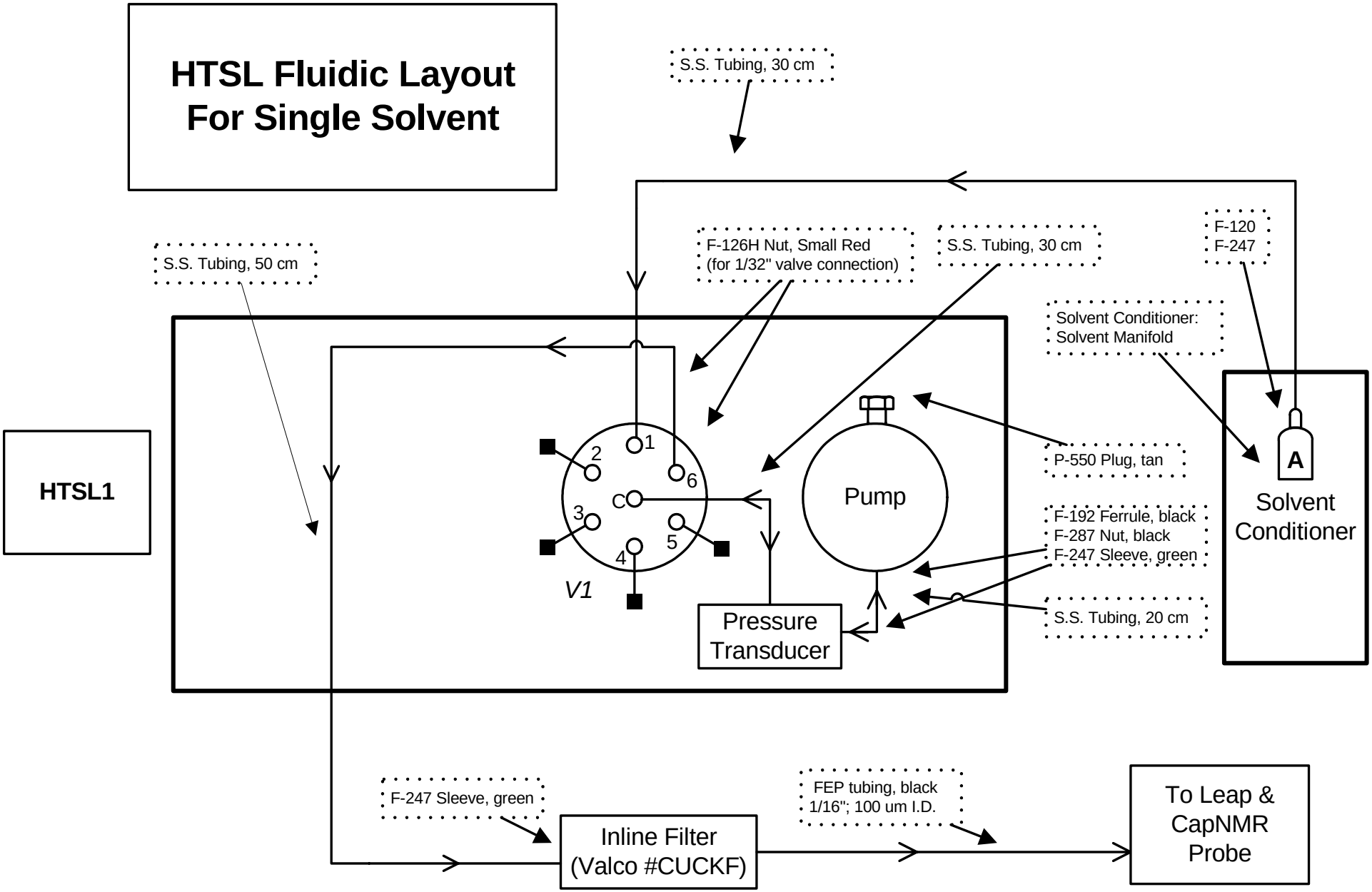
RevisionHistoy6.pdf attached.

Solvent Conditioner Fluidic Layout

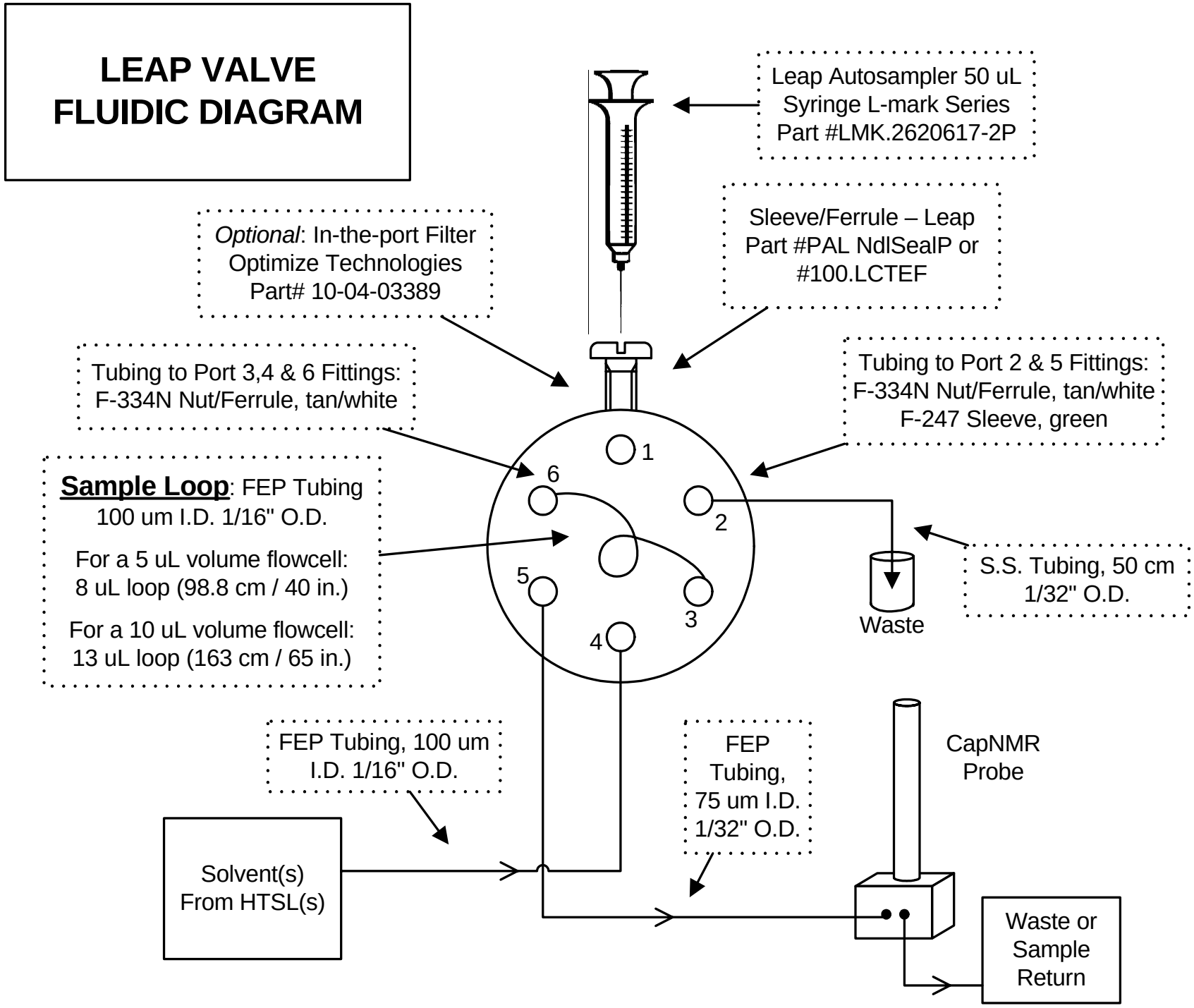


HTSL Fluidic Layout For Dual Solvent

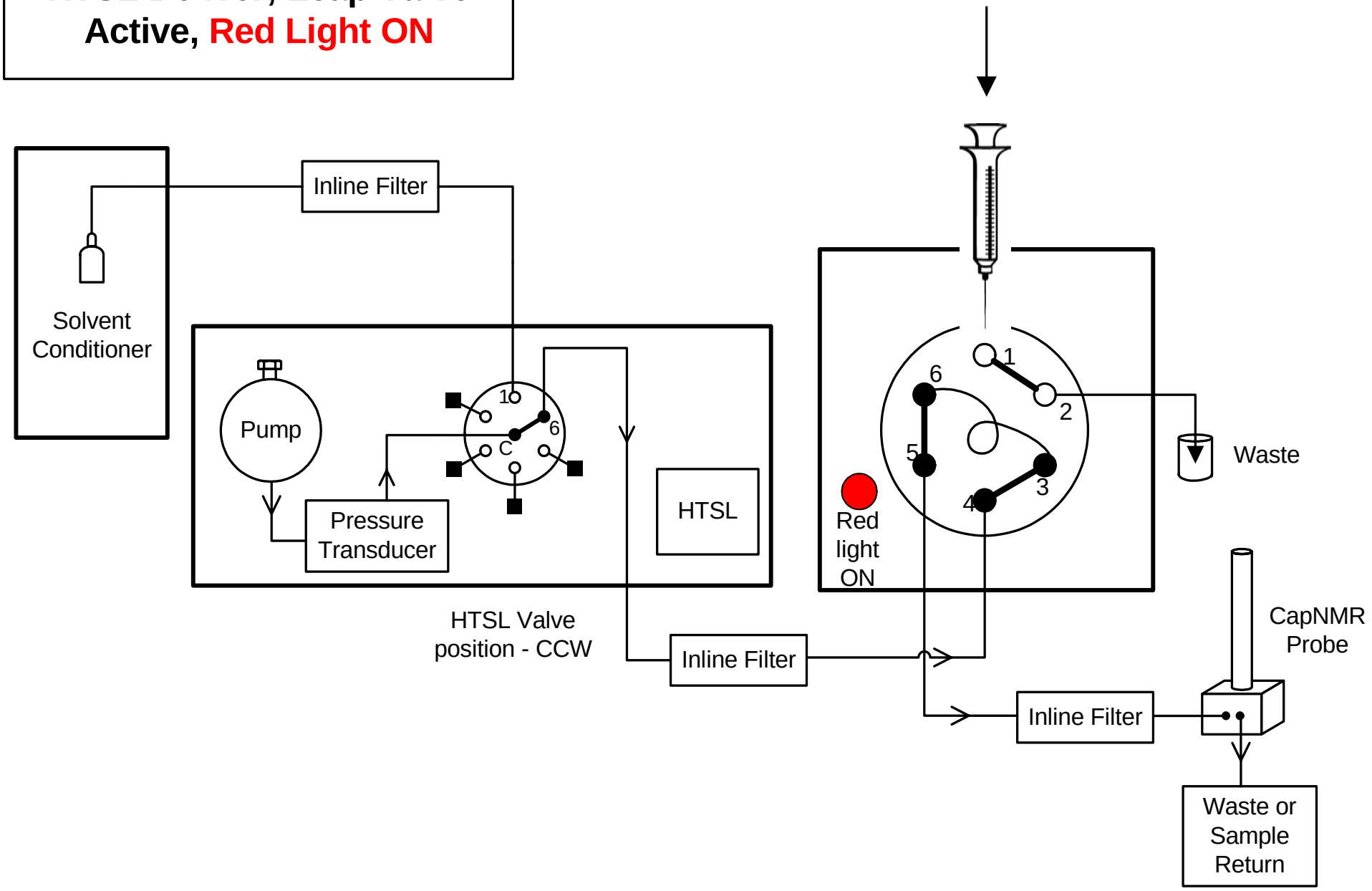



HTSL Fluidic Layout
For Single Solvent
S.S. Tubing, 30 cm
S.S. Tubing, 50 cm
F-126H Nut, Small Red
(for 1/32" valve connection)
S.S. Tubing, 30 cm
F-120
F-247
Solvent Conditioner:
Solvent Manifold
HTSL1
1
2
3
4
5
6
C
V1
Pump
P-550 Plug, tan
A
Solvent
Conditioner
F-192 Ferrule, black
F-287 Nut, black
F-247 Sleeve, green
S.S. Tubing, 20 cm
Pressure
Transducer
F-247 Sleeve, green
Inline Filter
(Valco #CUCKF)
FEP tubing, black
1/16"; 100 um I.D.
To Leap &
CapNMR
Probe

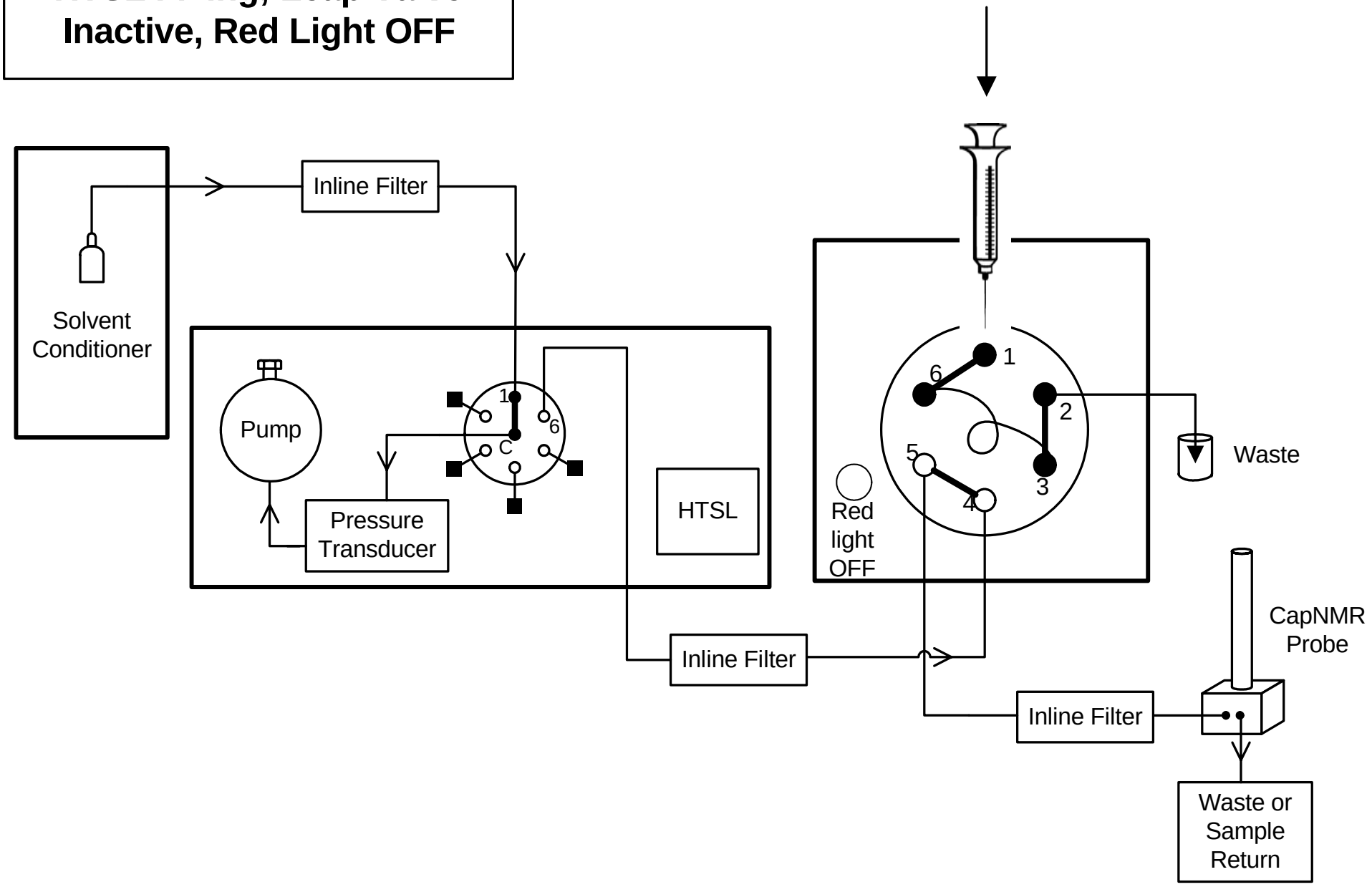
LEAP VALVE FLUIDIC DIAGRAM



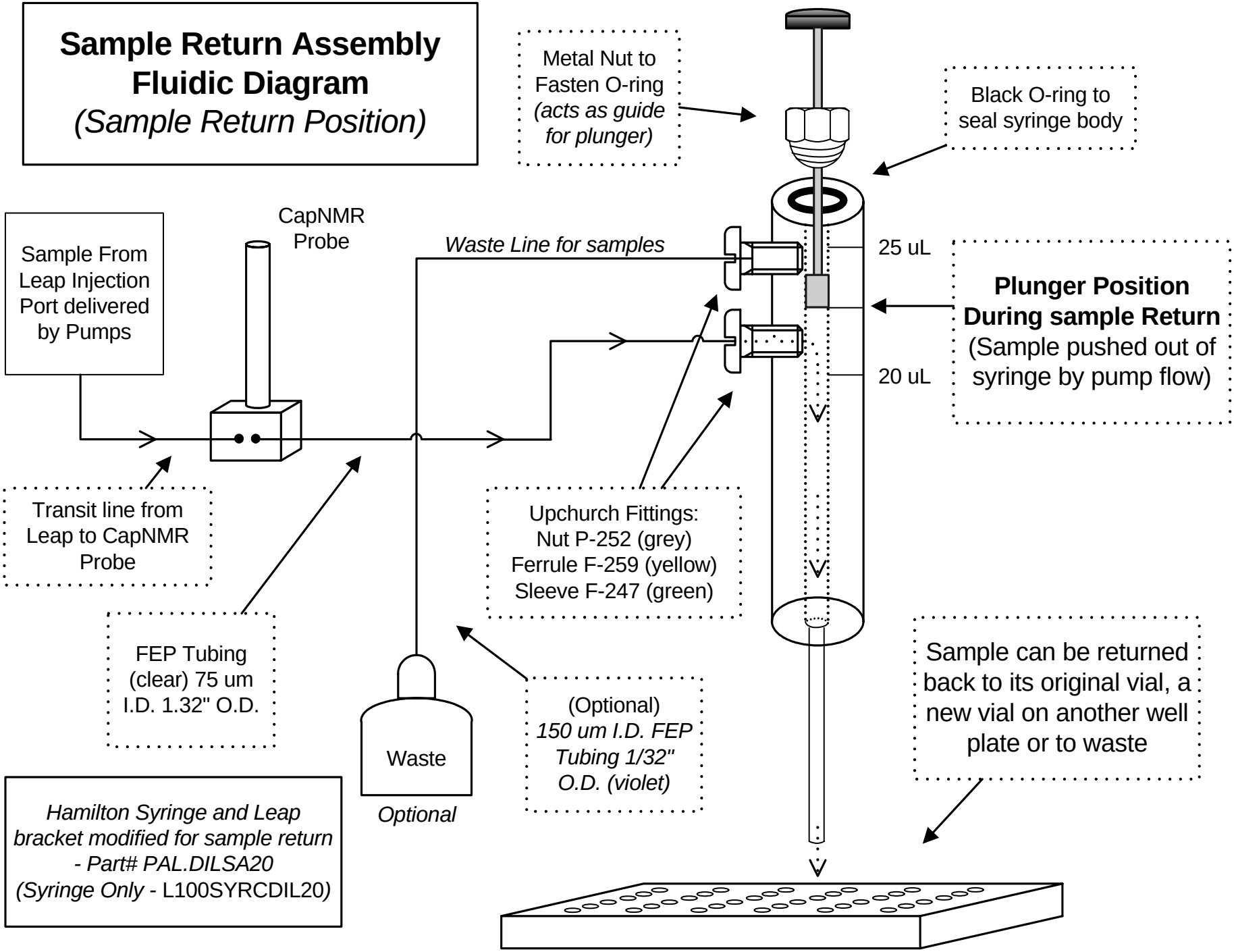
HTSL-LEAP Valve Diagram
HTSL Deliver; Leap Valve Active, Red Light ON



HTSL-LEAP Valve Diagram
HTSL Filling; Leap Valve Inactive, Red Light OFF



Sample Return Assembly Fluidic Diagram
(Sample Return Position)



Calibration Procedure for Delivery Volume on HTSL/Leap System

Preparing the HTSL

- Prime the HTSL if necessary. To control the HTSL, you may use the HTSL Control program within the Run Process of One-Minute NMR by selecting an HTSL from the pump menu.
- 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 (*see separate Leap directions elsewhere in this document*). This is the Active position, but the gameboy will indicate Inactive because that's what it *will* do if you hit the button.
- Set the HTSL to Fill at 80 μL/min, and Deliver at the μL/min flow rate you intend to use, such as 40 μL/min. Set the Delivery Volume to 95 μL.
- 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.

Preparing the NMR (Varian)

- Acquire a one-scan proton spectrum with the CapNMR probe from about 0-10 ppm using a reasonable point density. Set d1 to zero and set the acquisition time to a value where 1 scan = 1 μL.
- On a Varian system, with NT = 1, array NT to 100 uL worth of elements, with a zero increment between array elements. Check that the total time is correct as expected for the entire array. (i.e. array('nt',100,1,0) to setup a 100 single scan array)
    - For example, if the flow rate is set to 30 ul/min, then the goal would be to take 30 scans/min which would mean a 2 second scan would give easy math since 1 scan = 1 uL. To view array, 'wft dssh' will display the spectra in a time-base array.

Preparing the NMR (Bruker)

- Unfortunately, the Bruker system does not have an clean way of taking time sensitive data, as there are compiling delays of 1-2 seconds as well as I/O data saving delays with saving the data. As a result, the apparent time base is difficult to know, or is actually wrong.
- Several more difficult options involve using the lc2 pulprog that setup a pseudo-2D experiment with taking a false data in the second dimension, but there is a much simpler way that we have found to be very reliable. Please use this method, at least initially:
    - Inject the sample loop (see below for details) with the protonated version on your deuterated running solvent. Make sure the probe contains deuterated solvent and is locked in a normal manner on the solvent.
    - Open the HTSL Control program through the RunProcess and set the HTSL delivery volume to 95 μL.
    - Click on Run Pump to start delivering the protonated sample to the probe. However, start a stop-watch to time the lock level as it will drop to nearly zero as the protonated solvent enters the flow cell, and then return to its previous level as the protonated solvent is pushed out. Note the time from when the lock drops to zero to when it returns to normal, and the average of those two time values will be the on-flow delivery time. Use the flow rate to compute the corresponding on-flow Delivery Volume.

Preparing the Leap Autosampler

- Using the Leap gameboy, put the valve in the Red Light OFF state
    - When the red light is Off, it means the port is connected to the loop, then to Waste
    - When the red light is On, it means the port is connected to Waste only. So, Red = Waste.
    - Throwing the Leap Autosampler Valve
        - Menu
            - Utilities <enter>
                - Injector <enter> (hit <enter> again to select default injector)
    - Pressing Act Valve (F2) puts valve in red light Off position = injection port connected to loop
    - Pressing Deact Valve (F2) puts the valve in the red light On position = injection port not connected to loop, but connected to waste only
        - Home
- Using a 25-μL syringe (or larger) 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. *(Note: You may also use the Leap syringe, see Leap Optional notes for Leap Syringe Removal)*
- Load the loop manually via the port using the syringe and confirm that fluid exits the 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 to start the NMR acquisition (Varian); or, use the appropriate procedure for a Bruker spectrometer described above
- Use the 'wft dssh' command on the NMR (Varian) to display stacked spectra horizontally
    - Display one of the spectra ds(##) and zoom in on an appropriate spectral window to view just the peaks of the sample that were injected (generally, the solvent peak does not yield much information)
- You will see the flow profile appear (Varian) and the lock dip (Varian and Bruker)
- The maximum should be repeatable +/- one spectrum; do at least repeats. That maximum spectrum is also the number of μL to set the HTSL Delivery Volume to using Protyle, or the HTSL Control program of One Minute NMR (a tab in the Run Process window). This is the calibrated on-flow Delivery Volume for the HTSL.

Calibration of the Actual Stopped-Flow Delivery Volume

- The procedure above is used to determine the on-flow maximum; the corresponding volume can be used to determine the on-flow Delivery Volume. This is only an approximation of the desired Delivery Volume.
- The stopped-flow Delivery Volume is actually desired. Proceed to put some deuterated solvent in a vial or well plate, and spike it with a tiny volume of protonated solvent. Or, use a model compound dissolved in the deuterated solvent.
- Set the Delivery Volume in the Brain to the on-flow value determined above.
- Perform a One-Minute NMR run, and park the sample
- Measure the S/N of a distinct spectral feature in a systematic manner
- Repeat for a Delivery Volume that is 2 μL less than before.
- Continue this until it becomes evident which Delivery Volume yields the most S/N
- This is the correct Delivery Volume to set in the Brain and use in routine One-Minute NMR cycles.

Changing the Sample Loop

One Minute NMR is usually installed with a black sample loop, on the Leap valve between ports 3 and 6, which consists of 100-μm i.d., black FEP tubing, 1/32" o.d. and has an internal volume of 8.1 uL/meter.

Black FEP Tubing – 100 um Inner Diameter – 8.1 uL/meter
Clear FEP Tubing – 75 um Inner Diameter – 4.5 uL/meter

For a CapNMR probe with a 5-μL flow cell, a sample loop of 8 uL is recommended (40 inches (99cm) of the Black Fep Tubing) This loop accommodates loaded samples volumes of 8 μL or less. However, users with more then 8 uL of sample may also use a 10 uL loop is desired for overfilling the NMR flow cell – 49 inches (123 cm) of the Black Fep Tubing.

For a CapNMR probe with a 10-μL flow cell, a sample loop of 13 uL is recommended (65 inches (163cm) of the Black Fep Tubing) This loop accommodates loaded samples volumes of 13 μL or less. However, users with more then 15 uL of sample may also use a 15 uL loop is desired for overfilling the NMR flow cell – 73.5 inches (185 cm) of the Black Fep Tubing.

For instance, if you want to change from a 10-uL loop to a 15-uL loop, the following parts are employed. The tubing is Upchurch #1474, black FEP, which is a segment 10 feet long (also available in 20 feet). This tubing has an i.d. of 100 um, which yields 0.081 uL/cm (1 cm = 81 nanoliters). As a result, a length of 185 cm contains 15 uL. You may cut it to a length of 185 to 190 cm, and label it. Keep in mind that any volume loaded into the loop by the syringe of less than 15 uL is acceptable, but any excess sample will simple rinse the sample loop for a slight (1-3%) S/N boost.

When the loop is changed, use new fittings with the new loop. Use two new F-334N nuts, two new ferrules, F-142N (they come with the nuts) and two new green sleeves, F-247.

You may re-use the old loop. Store it carefully, and leave its fittings on it and in place. Squirt some solvent on the tubing tips to clean it just before re-use. It is best to flush the loop with solvent from the HTSL pumps before the 2nd end of the loop is connected to the Leap valve; this will prevent the 15 uL air bubble currently in the loop from being injected into the probe. Connect the loop first to Port 3 on the Leap valve, do one manual run (HTSL only), then connect the other end to Port 6. You'll see fluid drip out the tubing during the run; if not, perform one more manual HTSL run.

As a final check, perform an on-flow calibration for the new loop to determine the Delivery Volume to store in the HTSL.

Stepwise Hardware Installation for One-Minute NMR:

FEP & SS for the HTSL and Leap; FEP for the CapNMR Probe

The HTSL is plumbed to work with the Leap Autosampler, or as a stand-alone unit, the SS tube from Valve 1 (V1) to Valve 2 (V2) should be disconnected from V2. Use the special tool to unloosen the red nuts on V2.

Test the HTSL to make sure it is primed with the solvent of interest. Fluid should come out of the tube connected to Valve 1 Port 6 (V1P6) quickly after a Run command is made. If not, reprime with the solvent of interest. This should be quite easy, as the stainless steel solvent uptake tubing has a generous inner diameter of 500 μm.

For single solvent, the connection to valve 2 will be connected to the Leap valve. In dual solvent configuration, the tubing from both HTSL pump will first be directed through a solvent selection valve location on the master HTSL. This solvent selection valve connects to the Leap so by changing solvents, the enter flowpath may be pre-rinsed with the new solvent. Each Leap valve port should be squirted with acetone and dried with gas prior to any connections. See the diagram for what port to use.

The HTSL tubing from valve 2 should be connected to the Leap valve with a F-334N tan nut (includes ¼" hexagonal flat), clear telfon ferrule, and F-247 green sleeve. Rinse the fittings and tubing tip in advance with acetone to make sure they are clean. Blow away 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 special cutting blade (technic Knife by Olfa; ~$5). Make a square, 90 degree cut on a hard surface. Keep the blade clean, and use it exclusively for cutting FEP. All stored FEP tubing should be given clean cuts before use.

The loop will be FEP tubing mounted between LVP3 (Leap Valve Port 3) and LVP6. 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 acetone 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 SS tubing (500-μm i.d., 50-cm long) to LVP3 with a F-334N nut, ferrule, and green sleeve. 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----Clear FEP----M-135 filter----Clear FEP----Probe

If it is convenient, the union can be omitted.

Sample Return Option

This features involves returning the sample back to the original vial after NMR analysis, or to a different vial or even to waste in the sample is undesired. The Leap requires a special syringe and mounting plate in order to accommodate this feature.

In order to install the syringe and mounting bracket, position the modified Hamilton syringe into the blue mounting bracket by loosening the nut at the top of the syringe and taking the plunger and nut off. Place the syringe body into the bracket with the two side ports in the syringe facing left so it aligns with the bracket side ports for the tubing and fittings. Place nut and plunger through the top of the bracket into the top of the syringe and with the plunger inside the syringe body, tighten the nut (but not tight enough to restrict the motion of the plunger)

The Leap syringe definition must also be changed. This is done through:

Menu (F1)

- Setup (F3) <enter>
  - Objects <enter>
    - Syringes <enter>
      - Choose 20uL-SP *(SP means side ports = sample return)*

One Minute NMR Operating Tips

The sequence of NMR experiments must be listed in the desired order of execution in the "NMR Experiments" box on the main web page. Initially, experiments are probably listed in the order you created them. This is seldom the desired order of execution.

To change the execution sequence, highlight an experiment, then use the + or - buttons next to "Run Order" to move the experiment before or after another. The - button will move it up and the + button will move it down. You may have to highlight an experiment and move it several times to position it correctly.

Manual Priming Procedure for the HTSL in One Minute NMR

Automatic Pump Priming for Discovery Towers with Solvent Conditioners: *simply set the Solvent Conditioner program to Gas Blanket and take the fitting out of the top of the pump head. After several minutes, the solvent will flow through all the tubing and out of the top of the pump head. Once the pump is well flushed, screw the plug back into the top of the pump head and set the Solvent Conditioner program back to auto-blanket.*

Begin with the HTSL completely plumbed with the proper tubing. Solvent-handling tubing is all made from 1/32" o.d. stainless steel and has an i.d. of 500 μm to provide low flow resistance for solvent pick-up and priming.

Power on the HTSL. Watch for the LED panel to switch from emptying (yellow and green LEDs blink alternately), to filling (yellow LED blinking). The entire process takes 8-12 min. Once the Ready light comes on, and the pump is idle, start the RunProcess and connect with the HTSL Control program to the HTSL. Proceed to the Instrument Control tab, and hit "Pump Empty". Wait 4-6 min for this command to complete. Once empty, the pump is in the prime position with V1 set to connect to the solvent reservoir. Keep in mind the pump volume is 100 μL.

Rinse a 1-mL, Teflon Luer lock, air-tight, glass syringe with the deuterated solvent of interest. Attach the fitting (Upchurch #F-120) on one end of about a 5-inch segment of FEP tubing (cut with square tips using a razor), 1/16" o.d., and 0.030" i.d. (Upchurch #1522). Attach the F-120 to the syringe with an adapter (Upchurch #P-659), and place the syringe in the empty position. Fit the other end of the short tube with another F-120 fitting and screw it tightly into the side (upward) port of the pump head. Tighten snugly with the specialty black plastic wrench (Upchurch #P-298).

Place the solvent of interest in the reservoir and make sure the proper tube is submerged in the solvent. Pull firmly on the syringe and draw up the fluid, about 200 μL per pull, and wait about 10 sec between pulls. If the pump is completely cleared of bubbles, you should be able to pull up 200 μL or more without the appearance of a bubble, or an additional bubble. Look carefully at the translucent FEP tube to confirm the lack of a bubble in the flow path. Initially, the bubbles that appear are actually air inside the syringe pump, and will not collapse within the syringe barrel. However, if you pull quickly, bubbles form from cavitation, that is, a negative pressure within the syringe barrel. Some bubbles will originate from cavitation, which you should allow to collapse by influx of fluid.

Once you have stopped pulling up bubbles, make sure the remaining air bubble in the (vertically positioned) syringe is at the top of the barrel and check that the large FEP tube is filled with fluid. Unscrew the syringe from the P-659 PEEK adapter. Loosen it completely and keep the FEP tubing, still filled with solvent, in a vertical position. Gradually loosen the F-120 fitting at the syringe side port, and allow the solvent in the FEP tube to drain into the hole. Leaving that fluid in place, remove the F-120 fitting and screw the plug (Upchurch #P-550) into place in the side port. Tighten firmly with the special plastic wrench.

Next, using the Instrument Control tab again, hit "Pump Fill" and wait 4-6 min. While still not fluidically connected to the probe, check that the Fill and Delivery rates are both set to 25 μL/min. Set the Load Volume to 60 μL, save it, and perform a Run. You should see fluid emerge from the outlet tubing on the HTSL Valve (Port 3) which leads to the Leap valve. This tube from the HTSL valve to the Leap valve can be black, 100-μm i.d. FEP, or 500-μm i.d. stainless steel, whichever is convenient.

Should you overpressure the pump (P > 2500 psi), it will stall (both LEDs flash three times); just re-power, and choose a lower flow rate or shorter tubing length for the cycling steps. If the trial run completes successfully, and without any leaks detected, proceed to perform rinsing cycles. First, attach the transit tube to the Leap valve outlet tube (Port 5; usually 75-μm i.d. FEP). This will insure that the transit tube is rinsed prior to connection to the probe. Perform another, single trial Run with the Delivery rate set to 10-15 μL/min. If that trial completes successfully, set the number of cycles to 2 (Instrument Control tab), and press Empty/Fill. Verify that fluid again emerges from the outlet capillary. Allow the HTSL to complete this rinsing step of about 16 min.

Once these cyclic runs are complete, the HTSL should be in a primed state. With an appropriate length of tubing connected to the Leap valve (Port 5), and the Delivery Rate set to 15 μL/min, fluid should appear at the capillary outlet tip within ~30 sec of commencing a Run command of at least 20 μL of Delivery Volume. Next, perform a run (F = 15 μL/min; D = 90 μL; which is 6 min delivery time) while fluidically connected to the probe. The probe should be completely empty (flushed with acetone then air) before being rinsed with solvent. Note that the sample loop need not be loaded for any of the rinsing steps, as it is filled with solvent at all times regardless. The Leap valve should be rinsed in both positions to ensure that the loop and all port-to-port pathways are rinsed.

To verify priming, repeatedly load and inject a sample of the deuterated solvent spiked with protonated acetone (or any other suitable sample). A 1:1000 dilution of acetone in the solvent makes a nice sample of about 15 mM acetone-p_6. You can also use 10 mM sucrose in the solvent of choice. Load the Leap valve loop slowly with the 10-, 25-, or 50-μL syringe to ensure proper filling of the loop. Be sure to load the loop only when the pump LED indicates “Ready”, and the HTSL is idle, and the Leap valve Red light is OFF. You’ll need to throw the Leap valve yourself to the Red light ON position to put the loop on flow. Try an initial HTSL Delivery Volume of 60 μL, which is intentionally larger than that actual transit volume from sample loop to NMR probe. With a 1-scan NMR spectrum acquired every 4 sec, the maximum of the side-by-side spectra should appear in the same spectrum from injection to injection (Run to Run). If the reproducibility is close, but not perfect, perform another 90-μL run with the probe connected. Re-check the reproducibility, and if unacceptable, you will need to re-prime. If primed properly the first time, this is unlikely.

The pump should stay primed for an indefinitely lengthy period, as long as the HTSL solvent tube inlet tip is kept submerged in the reservoir solvent.

If the HTSL fluid is changed to a different solvent, you will need to rinse the pump with the new solvent, then re-prime as before. Once the priming procedure is understood, it takes only a few minutes, plus the pump empty and fill time, and rinsing the tubing and probe.

Site Checklist for One-Minute NMR

Intranet/Internet

- Cat5e cable with active Intranet or Internet connection to NMR console computer.
- Fixed domain name or IP address for NMR console computer
- Fixed IP address or domain name for One-Minute NMR computer, if remote web access to One-Minute NMR is desired. Protasis will configure the computer on-site.
- E-mail account dedicated to One-Minute NMR, to send alerts and completion notices. (Can use hotmail or other external e-mail server if desired)

Gas Supply (Discovery Tower Customers Only)

- 99.995% Helium (ultra high purity grade)
    - Regulated to 10 PSI (regulator not over 50 PSI)
    - From 1/16" outer diameter PEEK or stainless steel tubing
        1. Recommend Upchurch Part# 1533

Surface Area

- Bench top space approximately 5 feet wide by 3 feet deep
    - As close to magnet as possible while still outside the 5 gauss line
    - If placing unit on a cart, the cart should have a 500 lb load rating and be constructed from non-magnetic materials

Power

- Four (4) power outlets (or power strip) within 5 ft of bench top
    - Standard 120 V outlets
    - Total current capacity demand < 20 A
    - Approximate power ratings:
        1. Computer (stand-alone, non-Discovery Tower Customers): 300 W
        2. Computer (Discovery Tower Customers): 250 W
        3. Discovery Tower (includes all modules except computer): (300 W)
        4. HTSL (stand-alone, each): 115 W
        5. Leap autosampler: 150 W

Solvent

- Approximately 300 mL each of 2 to 3 desired deuterated solvents

System Maintenance

Leap Pal Valve

We recommend changing the rotor in the Leap valve every 3000 - 10000 actuations. It is time to change the rotor if parking becomes irreproducible or the valve leaks somewhere other than at a fitting in a port. If a blockage incident occurs within the valve, and is cured by cleaning the valve, it might be wise to also change the rotor, since it may have been damaged by the blockage material.

MicroLiter Analytical Part# L100LC26R
Valco Part# SSAC6W

Order from either vendor. A technical bulletin with detailed direction for changing this rotors (with color photos) is located at the technical support site microNMR.com (***See Technical bulletin N0700***) This is straightforward, though it must be performed carefully.

Leap Pal Needle Guide

Occasionally lubricate the slide rods of the Needle Guide with a silicone lubricant. If you ever hear any chattering when the Needle Guide moves up or down, lubricate the slide rods while simple multi-purpose synthetic-based oil with PTFE (i.e. "Super Lube®")

Changing Helium Gas Cylinder

- *Begin by closing the stopcocks at the bottom of the solvent conditioner door*. This will prevent the solvent that runs up to the Leap wash stations from draining out while the bottle is un-pressurized.
- Replace the gas cylinder tank
- Finish by opening the stopcocks at the bottom of the solvent conditioner door which will then allow flow to resume to the wash stations.

Refilling the Solvent Reservoir Bottles on the Solvent Conditioner

Begin by closing the stopcocks at the bottom of the solvent conditioner door. This will prevent the solvent that runs up to the Leap wash stations from draining out while the bottle is un-pressurized.

- Unscrew the bottle (turn the front of the bottle to the left to unscrew)
- Refill bottle to appropriate amount of solvent
- Reattach bottle with a firm seal
- Open the Solvent Conditioner program
    - Select appropriate COM port (generally COM2)
    - For the solvent channel that was just filled (Solvent A, B, C), select Sparge from the drop down menu which will open the vent and gas supply and purge the reservoir bottle of any atmospheric moisture that got it when the bottle was disconnected.
    - Click 'OK' at the bottom of the program to enable the Sparging and take a look at the solvent bottle. The helium should now be bubbling through the bottle.
    - After about 2 minutes or sparging, re-open the Solvent Conditioner program and select Gas Blanket from the drop down menu and Click on 'OK' at the bottom of the screen which will close the program and enable sparging.

- This will close the vent but leave the gas on which will bring the bottle back up to 5 PSI of pressure. Watch the tubing in the solvent and observe the rate of the helium bubbles. After 3-5 minutes, that bubbling rate should dramatically decrease (if not stop).
    - If the bottle continues to bubble after 10 minutes, make sure the bottle is tight and the seal is made around the bottle.
- After 5 minutes and once the bubbling has slowed, open the Solvent Conditioner program and select auto-blanket from the drop down menu and close the program.

This will leave the system with a pressurized bottle that has been purged of atmospheric air and will continue to auto-blanket at a rate determined by you (setting for auto-blanket are at the bottom on the screen; On Time and Off Time). Finish by opening the stopcocks at the bottom of the solvent conditioner door which will then allow flow to resume to the wash stations.

Saving Settings in the One-Minute NMR Brain *(Reference Only)*

Here are instructions on how to copy your One Minute NMR settings and save them for yourself, or send to someone else, perhaps for advice or troubleshooting.

In One Minute NMR, go to the Brain Web Page, then:

- Go to File …
    - Save As …
        - Declare file name and location
        - Choose file type Web Page, Complete
            - Save

- E-Mail the saved file to Protasis/MRM personnel if directed.
- Presently, this only works on the main Brain page General Settings. The page is not active, but just an image.

For other sections in the Brain:

- Do: Edit, Select All, Copy, then Paste into Word to capture the other parameters

Paste into a Word document, name, and save

One Minute NMR Standard Settings for a Dual-Solvent System

General Settings Section in the Brain

Settings To Edit:

Setting	Value	Description
Communication.CTC.COM	COM1	The serial port for communication with the CTC/LEAP liquid handler
Communication.Email.AlertRecipients	Bob Albrecht	Comma separated list of One-Minute NMR user names to send alerts to.
Communication.Email.FromAccount	b.albrecht@protasis.com	Account used to send mail to users.
Communication.Email.FromName	One-Minute NMR 1	The name of the One-Minute NMR system that is sending e-mail
Communication.Email.Host	protasis.com	The SMTP server for sending e-mail messages to users.
Communication.Email.LinkToMe	http://ht-nmr.com/OneMinuteNMR/main.aspx?RunEnable=True	Link to this One-Minute NMR system added to the bottom emails.
Communication.Email.Password	methods	Password for e-mail account that sends mail when authentication is required.
Communication.Email.SendAlerts	False	True if warnings and errors should be sent by e-mail.
Communication.Email.SendFinishedNotice	True	Set to True if One-Minute NMR sends messages about finished batches.
Communication.Email.SendPasswords	True	Set to True to e-mail passwords to users.
Communication.Email.Username	albrecht	The username when authentication is needed for sending e-mail.
Communication.HTSL.COM	COM4	The serial port for communicating with the HTSL
Communication.NMRIP	10.0.0.101	IP address or DNS of the NMR system
Database.RemovedSampleExpiration	24	The number of months after which removed samples are automatically purged
Database.Version	13	The version of the database schema used for automatic updates
HTSL.DeliveryRate	25	HTSL delivey rate in microliters per minute.
HTSL.DeliveryVolume	25	HTSL delivery volume.
HTSL.FillRate	15	HTSL fill rate in microliters per minute.
HTSL.Pressure	139	Current HTSL Pressure reading.
HTSL.PressureEnabled	true	True if the pressure transducer is enabled.
HTSL.PressurePercent	17	Percentage of maximum pressure.
HTSL.PressureUnit	PSI	Units for HTSL pressure. PSI or MPa.
HTSL.PressureUpdateInterval	3	Interval, in seconds to update pressure reading.
Load.CleanInjector1.cycles	1	Number of clean cycles
Load.CleanInjector1.delay	0	Fill delay in seconds
Load.CleanInjector1.ejectSpeed	5	Eject speed in microliters per second
Load.CleanInjector1.fillSpeed	5	Syringe fill speed in microliters per second
Load.CleanInjector1.rinseTime	1	Time to open rinse valve in seconds
Load.CleanInjector1.station	Wash1	Wash station to use for cleaning the injector
Load.CleanInjector1.volPercent	100	Percent of syringe to use for cleaning injector
Load.CleanInjector2.cycles	1	Number of clean cycles
Load.CleanInjector2.delay	0	Fill delay in seconds
Load.CleanInjector2.ejectSpeed	5	Eject speed in microliters per second
Load.CleanInjector2.fillSpeed	5	Syringe fill speed in microliters per second
Load.CleanInjector2.rinseTime	1	Time to open rinse valve in seconds
Load.CleanInjector2.station	Wash2	Wash station to use for cleaning the injector
Load.CleanInjector2.volPercent	100	Percent of syringe to use for cleaning injector
Load.CleanSyringe.post1.cycles	1	Number of cleaning cycles, first post-injection cleaning
Load.CleanSyringe.post1.delay	0	Pullup delay in seconds,first post-injection cleaning
Load.CleanSyringe.post1.ejectSpeed	5	Eject speed in microliters per second, first post-injection cleaning
Load.CleanSyringe.post1.fillSpeed	5	Fill speed in microliters per second, first post-injection cleaning
Load.CleanSyringe.post1.rinseTime	1	Time to open rinse valve in seconds
Load.CleanSyringe.post1.station		Wash station where the syringe is cleaned, first post-injection cleaning
Load.CleanSyringe.post1.volPercent	100	Percent of syringe to clean, first post-injection cleaning
Load.CleanSyringe.post2.cycles	1	Number of cleaning cycles, second post-injection cleaning
Load.CleanSyringe.post2.delay	0	Pullup delay in seconds,second post-injection cleaning
Load.CleanSyringe.post2.ejectSpeed	5	Eject speed in microliters per second, second post-injection cleaning
Load.CleanSyringe.post2.fillSpeed	5	Fill speed in microliters per second, second post-injection cleaning
Load.CleanSyringe.post2.rinseTime	1	Time to open rinse valve in seconds
Load.CleanSyringe.post2.station		Wash station where the syringe is cleaned, second post-injection cleaning
Load.CleanSyringe.post2.volPercent	100	Percent of syringe to clean, second post-injection cleaning
Load.CleanSyringe.pre1.cycles	1	Number of cleaning cycles, first pre-injection cleaning
Load.CleanSyringe.pre1.delay	0	Pullup delay in seconds,first pre-injection cleaning

Load.CleanSyringe.pre1.ejectSpeed	5	Eject speed in microliters per second, first pre-injection cleaning
Load.CleanSyringe.pre1.fillSpeed	5	Fill speed in microliters per second, first pre-injection cleaning
Load.CleanSyringe.pre1.rinseTime	1	Time to open rinse valve in seconds
Load.CleanSyringe.pre1.station	Wash1	Wash station where the syringe is cleaned, first pre-injection cleaning
Load.CleanSyringe.pre1.volPercent	100	Percent of syringe to clean, first pre-injection cleaning
Load.CleanSyringe.pre2.cycles	1	Number of cleaning cycles, second pre-injection cleaning
Load.CleanSyringe.pre2.delay	0	Pullup delay in seconds,second pre-injection cleaning
Load.CleanSyringe.pre2.ejectSpeed	5	Eject speed in microliters per second, second pre-injection cleaning
Load.CleanSyringe.pre2.fillSpeed	5	Fill speed in microliters per second, second pre-injection cleaning
Load.CleanSyringe.pre2.rinseTime	1	Time to open rinse valve in seconds
Load.CleanSyringe.pre2.station	Wash2	Wash station where the syringe is cleaned, second pre-injection cleaning
Load.CleanSyringe.pre2.volPercent	100	Percent of syringe to clean, second pre-injection cleaning
Load.GetSample.delay	1	Fill delay in seconds
Load.GetSample.fillStrokes	0	Number of fillVolume strokes of sample to rinse the syringe with
Load.GetSample.fillVolume	0	Microliters of sample for each rinse of syringe
Load.GetSample.InjectAfterRun	False	True to prevent loop load before previous run finished.
Load.GetSample.rate	2	Fill rate in microliters per second
Load.GetSample.volume	5.5	Sample volume in microliters
Load.GetSolvent.delay	1	Fill delay in seconds
Load.GetSolvent.location	Location for picking up push solvent	
Load.GetSolvent.speed	2	Push solvent fill speed in microliters per second
Load.GetSolvent.volume	0	Push solvent volume in microliters
Load.Injector.valve	CTC	Injector valve. CTC for Injector valve,or HTSL
Load.InjectSample.delay	1	Eject delay in seconds
Load.InjectSample.injector		LC Vlv1Name of injector
Load.InjectSample.rate	5	Eject speed in microliters per second
Load.InjectSample.volume	0	Volume to inject if different from GetSample.volume + GetSolvent.volume
Load.ReverseRinse.delay	2	Delay in secods after reverse rinse
Load.ReverseRinse.ejectSpeed	2	Microliters per second for eject
Load.ReverseRinse.fillSpeed	1	Microliters per second for reverse rinse
Load.ReverseRinse.volume	2	Number of microliters for reverse rinse
Load.ReverseRinse.waste	Waste	Waste port to use for reverse rinse
Macro.AfterRun		Macro after run.
Macro.Load.AfterInject		Macro after inject.
Macro.Load.BeforeClean		Macro before the first syringe cleaning.
Macro.Load.BeforeGetSample		Macro after syringe cleaning, before getting sample.
NightSample.Begin	18:00	The 24-hour time after which night samples can start
NightSample.End	6:00	The 24-hour time after which night samples cannot start
NMR.CommandFile	C:\Bruker\XWIN-NMR\prog\xwin_cmd.txt	File for sending commands to the NMR
NMR.Disk	C:\	The disk where NMR data are stored
NMR.ErrorLevel	Soft Error	Varian error level. Warning, Soft Error, or Hard Error (acqstat[1]=14,13,15)
NMR.Interface	Varian	The NMR Interface to use. (Varian or Bruker.Windows)
NMR.LockTable	autolock	Table to use for initial lock settings on Varian system
NMR.ProbeFile	capnmr	Probe file to use for Varian spectrometers.
NMR.PulseDelay	1	Number of seconds of delay after each pulse
NMR.ResponseFile	C:\Bruker\XWIN-NMR\prog\cmd_response.txt	File for getting status back from the NMR
NMR.ScriptStatus	nmrstatus	The command sent for monitoring status on the Varian system. (showstat)
NMR.ScriptTemplate	autorun	Basic script used to run all samples.
NMR.ShimFile	shim	File for shimming the probe.
NMR.ShimFileExtension	mrm	Varian extension to add to solvent for shim file e.g. d2omrm
NMR.TemperatureEquilibrationTime	0	Time in seconds to wait after sample is loaded before lock
ProbeClean.Countdown	0	Countdown for probe cleaning.
ProbeClean.Duration	60	Seconds to leave solution in probe.
ProbeClean.Enabled	False	True to activate Probe cleaning.
ProbeClean.Frequency	1	How often to clean the probe. 0=never, 1=every, n=every nth
ProbeClean.Index	1	Vial index for wash solution.
ProbeClean.Tray	Tray01	Vial tray for wash solution.
SampleReturn.CleanSyringe.pre1.cycles	1	Number of cleaning cycles, first pre-return cleaning
SampleReturn.CleanSyringe.pre1.delay	0	Pullup delay in seconds, first pre-return cleaning
SampleReturn.CleanSyringe.pre1.ejectSpeed	5	Eject speed in microliters per second, first pre-return cleaning
SampleReturn.CleanSyringe.pre1.fillSpeed	5	Fill speed in microliters per second, first pre-return cleaning

SampleReturn.CleanSyringe.pre1.rinseTime	1	Time to open rinse valve in seconds
SampleReturn.CleanSyringe.pre1.station		Wash station where the syringe is cleaned, first pre-return cleaning
SampleReturn.CleanSyringe.pre1.volPercent	100	Percent of syringe to clean, first before sample return.
SampleReturn.CleanSyringe.pre2.cycles	1	Number of cleaning cycles, second pre-return cleaning
SampleReturn.CleanSyringe.pre2.delay	0	Pullup delay in seconds, second pre-return cleaning
SampleReturn.CleanSyringe.pre2.ejectSpeed	5	Eject speed in microliters per second, second pre-return cleaning
SampleReturn.CleanSyringe.pre2.fillSpeed	5	Fill speed in microliters per second, second pre-return cleaning
SampleReturn.CleanSyringe.pre2.rinseTime	1	Time to open rinse valve in seconds
SampleReturn.CleanSyringe.pre2.station		Wash station where the syringe is cleaned, second first pre-return cleaning
SampleReturn.CleanSyringe.pre2.volPercent	100	Percent of syringe to clean, second before sample return.
SampleReturn.EjectSpeed	5	Syringe eject speed in microliters/second.
SampleReturn.Enabled	False	Set to True if sample return is enabled and plumbed.
SampleReturn.LoadedSample	399	The ID of the last loaded sample so it can be returned.
SampleReturn.Penetration	2000	Micrometers of needle penetration for sample return.
Settings.AllowDelete	False	True if deletes are allowed. (DANGEROUS)
SolventSelect.BunchNightSamples	True	Run night samples with the same solvent together.
SolventSelect.BunchSamples	True	Runs samples with the same solvent together.
SolventSelect.CurrentSolvent	D2O	The current solvent used by the system. For detecting changes.
SolventSelect.FlushCycles	15	Number of flush cycles.
SolventSelect.FlushEmptySpeed	25	Flush empty speed in microliters per minutes.
SolventSelect.FlushFillSpeed	25	Flush fill speed in microliters per minute.
SolventSelect.FlushVolume	100	Volume in microliters to flush pump.
SolventSelect.PrioritySwitch	False	Priority samples can force a solvent switch.
SolventSelect.ProbeFlushCycles	3	Number of probe flush cycles.
SolventSelect.UniversalSolvents	Acetone,DMSO	List of universal solvents.
SolventSelect.ValveWastePort		The port to eject waste solvent to. Default probe port.
System.Paused	False	Set to True when the system is paused
System.SampleCounter	0145	Counts the number of samples that have run.
System.Status	>Sample Acquisition Done >NMR Run Complete >Done Loading >Loading Sample: Sat_28 >Finished Load Sequence >Go Home >Reverse Rinse >Startng NMR Run	Current system status message.

Available Solvents Section in the Brain

Settings To Edit:

Solvent	Pump	Sparge	Wash	Vol. Full (mL)	Vol. Remaining (mL)	Polar (Yes/No)
D2O	HTSL1		Wash1	0	0	yes
DMSO	HTSL2		Wash2	0	0	no

Name	Master Port	COM	Delivery Rate	Fill Rate		Volume
HTSL1	HTSL1	1	COM1	25	25	5
HTSL2	HTSL1	2	COM4	25	15	5

Pumps Section in the Brain

Settings To Edit:

Name	Master Port	COM	Delivery Rate	Fill	Rate	Volume
HTSL1	HTSL1	1	COM1	25	25	5
HTSL2	HTSL1	2	COM4	25	15	5

Available Experiments Section in the Brain

Settings To Edit:

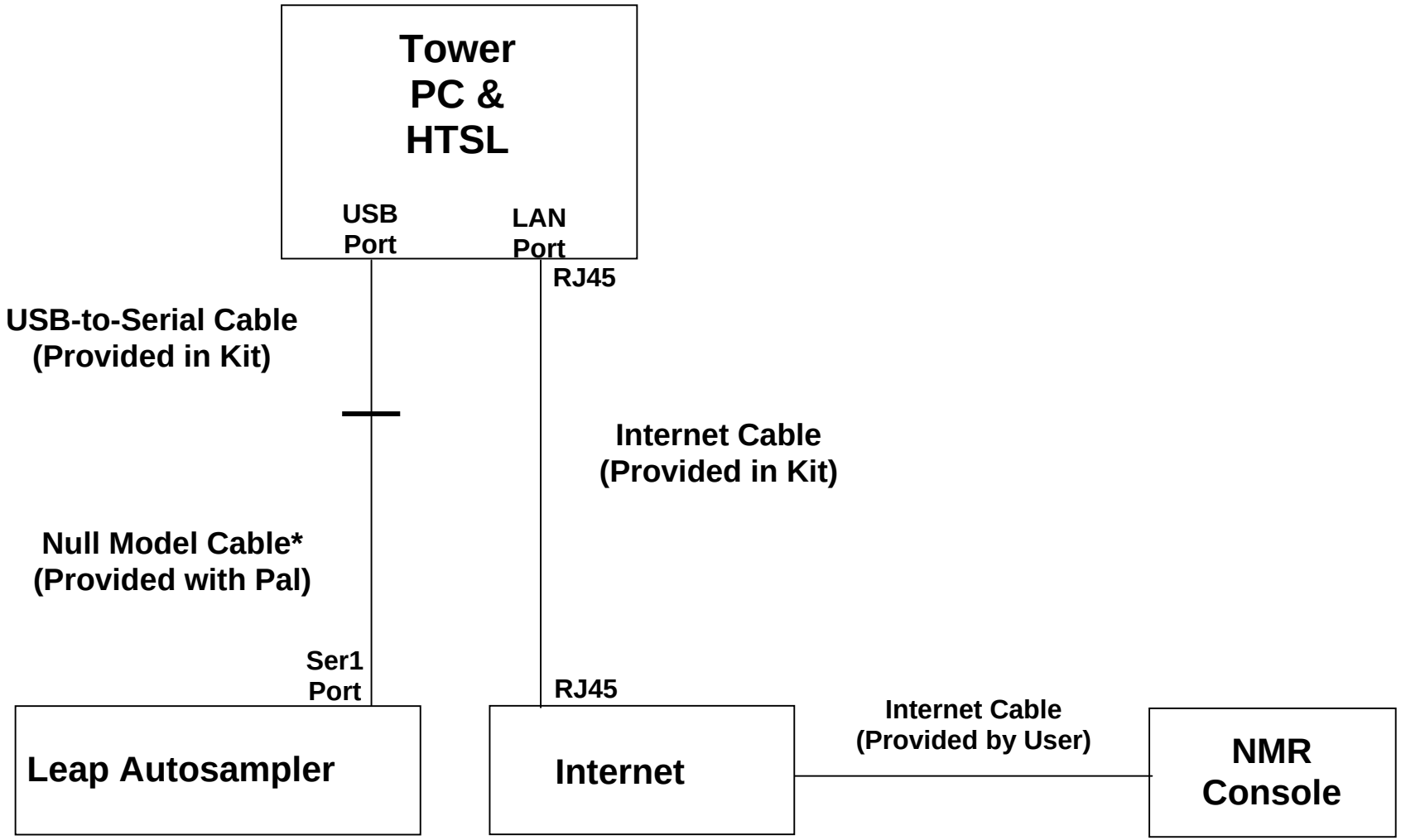
Exp. Name	Nucleus	Exp. #	Num. Scans	Spectral Width	Spectrum Center	ProcessFile
1dproton	PROTON	4	1	10	0	
carbon1	PROTON	4	256	10	0	
scout1d	PROTON	4	1	10	0	
wet1d	PROTON	4	16	10	0	

Import Conversion

Settings To Edit:

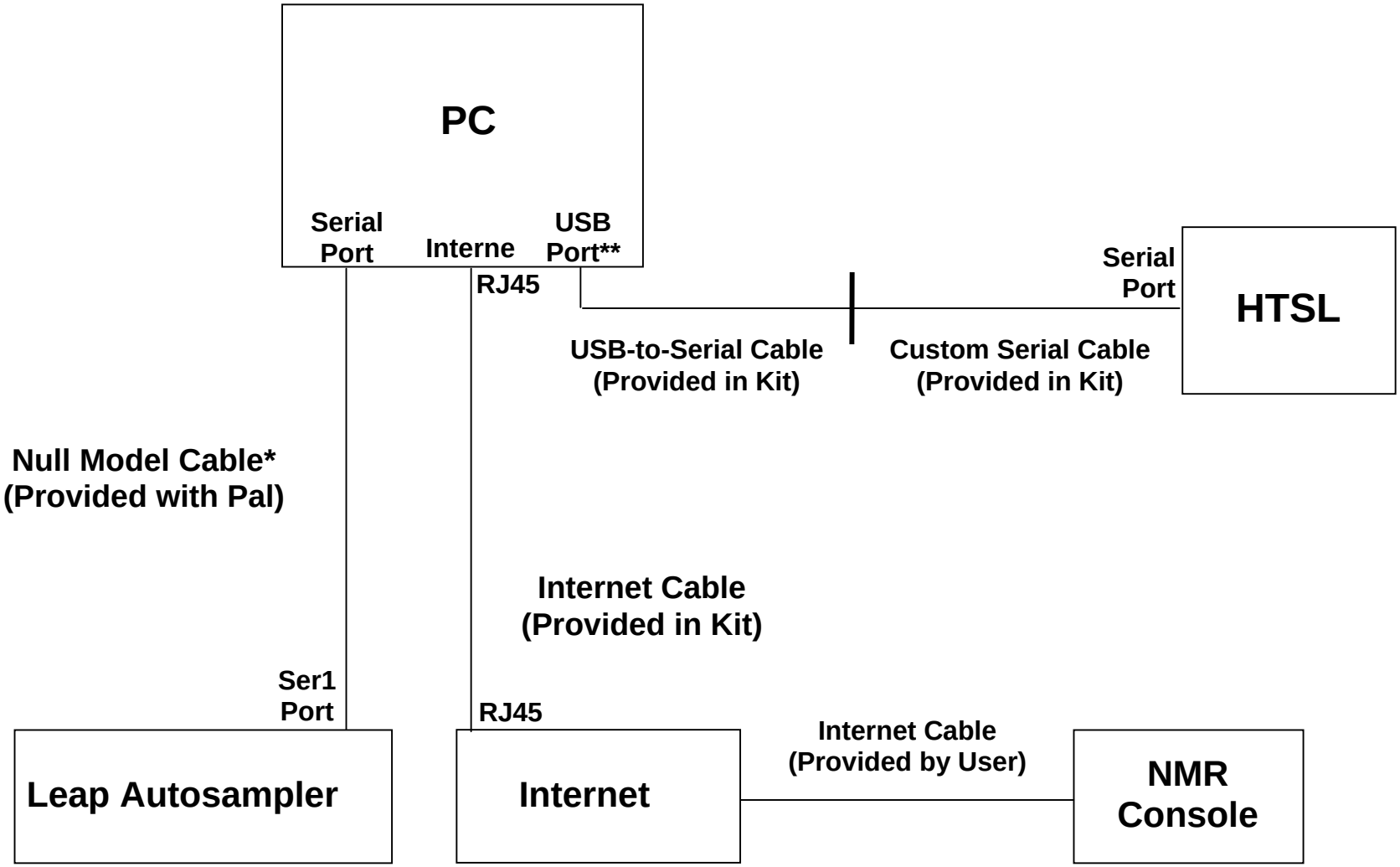
Import ID	CSV Field	Samples Table Field
default	Experiment	Experiments
default	Index	VialIndex
default	SampleID	FileName
default	Solvent	Solvent
default	Title	SampleID
default	UserID	Username

One-Minute NMR Cable Connections
Tower Configuration



*Not labeled as received from Leap

One-Minute NMR Cable Connections
HTSL Configuration



*Not labeled as received from Leap
**Serial port use ok if available

Cable Specifications
Provided by Protasis/MRM

In Kit

USB-to-Serial Cable:
- **Sewell SW-1301 is preferred; comes with CD**

Custom Serial Cable:
- **9-pin serial cable modified by Protasis/MRM**
- **Cable has just pins 2, 3, and 5; OR all pins except 4 & 7**
- **CablesNMor #P22123 (10-ft) is preferred**

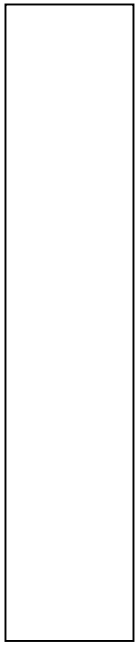
Internet Cat5e Cables (RJ45 connector):
- **25-foot cable; User may substitute cable of desired length**
- **3-foot cable provided for Tower configuration only (inside-the-Tower use)**

In Installer Toolbox Only (Standby)

- **Standard internet coupler**
- **10-foot internet cable (Cat5e)**

Port Connections
Leap Autosampler, Tower, HTSL

Leap Autosampler (rear)



Ser 1

Ser 2

Ser 3 (Gameboy)

LED Buzzer

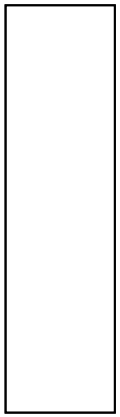
Power

Wash Station

Interface

Aux (valve)

HTSL (rear)



Power

Serial Port

Liquid Handler

Remote Control

Tower (rear)