

**MicroVAST
Interface and
Operations Manual
(FEP Tubing Version)**

16 December 2005

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Table Of Contents

	<u>Page</u>
MicroVAST Spare Parts List	3
Gilson Current Parameters at MRM for MicroVAST	4
Gilson Parameters Template for MicroVAST	5
Description of Terminology in Gilson Enter Window	6
MicroVAST Installation	8
Fluidic Hardware Modifications	8
Stopped-Flow Time Calibrations	8
Introduction to MicroVAST Software	9
Installation Procedures for MicroVAST Software	10
MicroVAST Installer	10
MicroVAST Software Installation Notes	12
Manual MicroVAST Software Installation (not recommended)	14
Testing MicroVAST	15
VAST to MicroVAST Hardware Conversion	16
Gilson 215 Operational Notes	16
MicroVAST Layout with Gilson and HTSL	18
HTSL Fluidic Valve Diagram: Status HTSL Deliver	19
HTSL Fluidic Valve Diagram: Status HTSL Refill/Load Loop/Parked	20
HTSL 1100 Fluidic Layout	21
Automated Flow Injection: MicroVAST Basic Operation	22
Troubleshooting	23
Gilson/HTSL Cable Connection Scheme	24
TCL Code and the Varian gilson File	25

MicroVAST Spare Parts List

<u>Item</u>	<u>Quantity</u>	<u>Vendor & Part #</u>
PEEK tubing; 1/16" o.d., i.d. 0.030", 5 ft; green	2	U; 1533
In-line filter (4.0 µL) for Gilson & HTSL solvent; metal	2	U; A-314
Replacement frit for A-314; tan	1 (10-pk)	U; A-100x
Small nut and ferrule for above filters; black	1 (10-pk)	U; F-195x
Upchurch 1/16" o.d. sleeve for capillary; green	1 (10-pk)	U; F-242x
Nuts for Gilson syringe valve and z-arm; gray	1 (10-pk)	U; P-252x
Ferrules for Gilson syringe valve and z-arm; yellow	1 (10-pk)	U; P-259x
Large nut and ferrule for Gilson port connection; black	1 (10-pk)	U; F-287x
Filter for Gilson port, 2-µm screen	2	V; CFE-S2
Union for filter, including nuts and ferrules	2	V; CUCKF
Syringe tip filters for bulk solvent filtration	1 (100-pk)	U; B-100x
Fused silica capillary, 150/360, 2 meters	1	U; FS-115
PEEK tubing cutter	1	U; A-350
Micro Rinse Station, PEEK, custom design from MRM only	1	M; RS215P
Solvent Bottles	2	M; Free
Gilson Micro Needle	1	G; 2507252
Gilson Micro Injection Port	1	G; 270728
Gilson 100 µL Syringe	1	G; 25025341
Gilson Injection Port Seal	1	G; 2707273
HTSL Modification for connection to pump		
Nut and ferrule combination	1 (10-pk)	U; F-331Nx
Sleeve; green FEP	2	U; F-247

U = Upchurch (1-800-426-0191); x denotes 10-pack

V = Valco (1-800-367-8424)

M = Protasis/MRM in Illinois (1-217-351-4359)

G = Gilson at 1-800

Gilson Current Parameters at MRM for MicroVAST

Fluidic Parameters: Volumes in μL ; Flow in mL/min

Sample Definitions

Probe Volume (μL)	<u>14</u>	Probe Slow Volume	<u>0</u>
Sample Volume (μL)	<u>10</u>	Probe Slow Rate	<u>0</u>
Push Volume (μL) _{computed}	<u>4</u>	Probe Fast Rate (mL/min)	<u>0.1</u>
Keep Sample	<u>No</u>	Sample Extra Volume	<u>0</u>
Number of Rinses	<u>1</u>	Rinse Extra Volume (μL)	<u>50</u>
Rinse Delta Volume (μL)	<u>0</u>		

Additional Parameters

Sample Well Rate (mL/min)	<u>1</u>	Delay HTSL Cycle (msec)	<u>HTSL Delivery time + 5 sec</u>
Sample Height	<u>0</u>	Mix Time	blank
Sample Depth	<u>NOSEEK</u>	Mix Flow Rate	blank
Needle Rinse Vol. (μL)	<u>100</u>	Mix Height	blank
Needle Rinse Rate (mL/min)	<u>1</u>		

Rack Definition (typical)

Rack number	205h
Zone	1: 12x8
Rack definition	left and back
Pattern	Z

Main Control

Air Valve	<u>off</u>	Plunger	<u>up</u>
Syringe Volume (μL)	<u>100</u>		
Arm Z scale (mm)	<u>125</u>		

Calibrations (Positional; in 0.1 mm)

Rinse	X	<u> </u>			
	Y	<u> </u>			
	Z	<u> </u>	settable; later unreadable		
Inject	X	<u> </u>			
	Y	<u> </u>			
	Z	<u> </u>	settable; later unreadable		
Rack	X	<u> </u>			
	Y	<u> </u>			
	Z	<u> </u>	settable; later unreadable		

HTSL Parameters

Solvent	<u> </u>
Delivery rate ¹ ($\mu\text{L}/\text{min}$)	<u>10</u>
Loop volume ¹ (μL)	<u>9</u>
Fill rate ¹ ($\mu\text{L}/\text{min}$)	<u>20</u>
Stop volume (μL) _{calibrated}	<u> </u>
Port link volume ² (μL)	<u>2+2 = 4</u>

¹Delivery rate, Loop volume, and Fill rate are all adjustable, but these are reasonable values

²Volume of tubing from the loading port to HTSL Valve 2

Gilson Parameters Template for MicroVAST

Fluidic Parameters: Volumes in μL ; Flow in mL/min

- Entries that are empty can be changed by the user
- Entries that have a zero, other entry, or say “blank” should remain unused

Sample Definitions

Probe Volume (μL)	<u> </u>	Probe Slow Volume	<u> 0 </u>
Sample Volume (μL)	<u> </u>	Probe Slow Rate	<u> 0 </u>
Push Volume (μL) _{computed}	<u> </u>	Probe Fast Rate (mL/min)	<u> 0.1 </u>
Keep Sample	<u> No </u>	Sample Extra Volume	<u> 0 </u>
Number of Rinses	<u> </u>	Rinse Extra Volume (μL)	<u> </u>
Rinse Delta Volume (μL)	<u> 0 </u>		

Additional Parameters

Sample Well Rate (mL/min)	<u> </u>	Delay HTSL Cycle (msec)	<u>HTSL Delivery time + 5 sec</u>
Sample Height	<u> 0 </u>	Mix Time	blank
Sample Depth	<u>NOSEEK</u>	Mix Flow Rate	blank
Needle Rinse Vol. (μL)	<u> 100 </u>	Mix Height	blank
Needle Rinse Rate (mL/min)	<u> 1 </u>		

Rack Definition (typical)

Rack number	205h
Zone	1: 12x8
Rack definition	left and back
Pattern	Z

Main Control

Air Valve	<u> off </u>	Plunger	<u> up </u>
Syringe Volume (μL)	<u> 100 </u>		
Arm Z scale (mm)	<u> 125 </u>		

Calibrations (Positional; in 0.1 mm)

Rinse X
 Y
 Z settable; later unreadable

Inject X
 Y
 Z settable; later unreadable

Rack X
 Y
 Z settable; later unreadable

HTSL Parameters

Solvent
 Delivery rate¹ ($\mu\text{L}/\text{min}$) 10
 Loop volume¹ (μL) 9
 Fill rate¹ ($\mu\text{L}/\text{min}$) 20
 Stop volume (μL)_{calibrated}
 Port link volume² (μL) 2+2 = 4

¹Delivery rate, Loop volume, and Fill rate are all adjustable, but these are reasonable values

²Volume of tubing from the loading port to HTSL Valve 2

Description of Terminology in Gilson Enter Window

These nine terms are listed in the order they appear in the Gilson window on the Varian console. They are not listed in the order they occur during a run.

All volumes are in μL ; all flow rates are in mL/min .

Probe Volume Total volume dispensed by the Gilson syringe into the HTSL port immediately after the solvent and sample are picked up.

Sample Volume The volume of sample in a well picked up by the Gilson needle.

Push Volume The volume of solvent drawn up by needle prior to picking up a sample.

Number of Rinses The number of times the needle is rinsed with solvent while in the loading port.

Probe Fast Rate The flow rate for the loading of the HTSL loop. The Probe Volume is loaded at this rate (used to load the sample loop). The Rinse Extra Volume is dispensed at this rate (used to rinse the port and loop).

Rinse Extra Volume The volume of solvent rinsed through the HTSL loading port and loop, to waste at the Probe Fast Rate

Sample Well Rate The flow rate at which solvent and sample is picked up by the Gilson needle.

Needle Rinse Volume The volume of solvent drawn in from the solvent reservoir and rinsed through the needle into the rinse station insert.

Needle Rinse Rate The rate at which the Needle Rinse Volume is drawn up from the solvent reservoir and dispensed into the rinse station insert.

The sequence of events is as follows:

- Gilson needle moves to well
- Gilson syringe pulls up the Push Volume from the Gilson solvent reservoir at the Sample Well Rate
- Gilson syringe pulls up the Sample Volume from the well at the Sample Well Rate

- Needle moves to port
- Gilson syringe dispenses the Probe Volume from the needle into the port at the Probe Fast Rate
- Built-in 9-sec pause before HTSL is triggered; fluidic relaxation time
- HTSL delivers, parks analyte (and begins to refill)
 - After the Delay HTSL Cycle time, NMR data is acquired
 - This step is not completed before the remaining rinsing steps are begun
- Gilson syringe pulls up the Rinse Extra Volume from its solvent reservoir at the Needle Rinse Rate
- Gilson syringe dispenses the Rinse Extra Volume at the Probe Fast Rate through the port and loop to waste (while HTSL is refilling)

- The syringe draws up $50 \mu\text{L}$ of solvent (syringe valve in solvent position) and expels it through the needle (syringe valve in needle position), which has remained in the port; the port is rinsed. The loop is not rinsed here; it is likely still on-flow delivering sample to the NMR probe

- The syringe pulls up 50 μL while the needle is still in the port (syringe valve in needle position). *This is the Reverse Rinse.* (Again, the loop is not rinsed; it is likely still on-flow delivering sample to the NMR probe.)
- The needle goes to the Rinse station and the syringe expels this Reverse Rinse solvent (syringe valve in needle position)
- Gilson syringe pulls up the Needle Rinse Volume from its reservoir at the Needle Rinse Rate
- Gilson syringe dispenses Needle Rinse Volume through needle into Rinse Station at the Needle Rinse Rate
- Cycle ends or repeats

Flow Rate Summary – Standard Settings

Needle Rinse Rate = 1 mL/min = 1000 $\mu\text{L}/\text{min}$ (used for Needle Rinse)
 Probe Fast Rate = 0.1 mL/min = 100 $\mu\text{L}/\text{min}$ (used for Reverse Rinse; Port Rinse, and Sample expulsion)
 Sample Well Rate = 1 mL/min = 1000 $\mu\text{L}/\text{min}$ (used for Sample and Solvent pick-up)

<u>Event</u>	<u>Sample and Solvent</u>	<u>Port Rinse</u>	<u>Reverse Rinse</u>	<u>Needle Rinse</u>
Pick-up	1 mL/min	1 mL/min	0.1 mL/min	1 mL/min
Expulsion	0.1 mL/min	0.1 mL/min	0.1 mL/min	1 mL/min

MicroVAST Installation

Fluidic Hardware Modifications

Refer to the diagram for details on fluidic component specifications.

- Install sample loop of proper volume (usually 9 μL) between ports 1 and 4 of Valve 2 on the HTSL. Usually, this is 49.5 cm of 150/360 capillary.
- Place HTSL on the right, rear of the Gilson bed, and fasten it down with its Protasis mounting bracket.
- Prime the HTSL with the solvent of interest (see HTSL Manual for details). Be sure the pump is in the Empty position when priming, and when priming is complete, restore the pump to the Full position. Perform some 90- μL runs to crush and flush any bubbles that might linger in the pump or flow path.
- Mount the Gilson microport on the HTSL mounting bracket (center hole is fine).
- Install fluidic link between port 5 on V2 of the HTSL and the Gilson microport. Use two 11-cm segments of 150/360 capillary, and include the Valco filter between the segments. Don't connect the capillary to the microport at this time.
- Install the solvent uptake tube for the Gilson syringe valve (pressure limit 120 psi).
- Install the solvent tube between the Gilson syringe valve, to the filter, and to the micro needle.
- Replace the Rinse Station insert with the PEEK micro insert.
- Make a sample of methanol- p_4 diluted 1:1000 (about 15 mM) in the solvent of interest, preferably using solvent from the HTSL solvent uptake bottle.
- Using a 250- μL syringe, push some of the 15 mM MeOH- p_4 through the link, into the sample loop, and overflowing out the waste capillary. Proceed to calibrate the HTSL for stopped-flow. Try a delivery rate of 10 $\mu\text{L}/\text{min}$, a fill rate of 20 $\mu\text{L}/\text{min}$, and NMR single-scan data acquisition every 6 sec = 0.1 min, for a total of 5 min.
- Cable the HTSL to the Gilson 215 with the special, provided cable.
- Install the MicroVAST tcl code and the MicroVAST gilson program (see below).
- Lastly, after all testing, install the Gilson micro needle and calibrate the x,y,z position of the rinse station, port, and well plate. Write down the values, and save them in the software.

Stopped-Flow Time Calibration

Prime the HTSL with the desired solvent and calibrate the time to deliver a sample from the loop to the CapNMR™ flow cell. Use a sample of methanol- p_4 diluted 1:1000 (about 15 mM) in the deuterated solvent of interest. Take a solvent sample from the solvent reservoir to match the HOD content of solvent and sample.

Cable the HTSL to the Gilson for MicroVAST operation.

Introduction to MicroVAST Software

Six files need to be replaced to perform MicroVAST. The five tcl (tool command language) files are:

- get.tcl (a required blank file)
- retrieve.tcl (a required blank file)
- inject.tcl
- put.tcl
- wash.tcl

A tcl file is created by saving a blank text document with the correct name. The location for the tcl files is /vnmr/asm/tcl/filename.tcl. Also, the additional file called:

gilson

will create a re-labeled box in the gilson window on the console to allow easy entry of the fluidic delivery time plus a delay. The file named gilson is located at /vnmr/tcl/bin/filename.

Roboproc and gilalign are available in new versions, and may be replaced if needed to make the Gilson 215 operate properly under VAST control. Keep in mind that these programs have no explicit version numbers. You can download the latest versions from the Varian web page. If Roboproc and gilalign are updated, the permissions must be changed to allow execution of them. The gilalign program is located in /vnmr/bin/. Roboproc is located at /vnmr/acqbin/filename.

To summarize, replacement of the five tcl code files is required for MicroVAST. The gilson file replacement is highly recommended, and required to yield a gilson window similar to the ones depicted in this document. The gilalign and Roboproc programs should remain unchanged unless a problem arises in operating the Gilson.

Installation Procedures for MicroVAST Software

It is required to begin with a working Gilson 215 and VAST system. For conversion to MicroVAST, the Gilson 215 will need replacement of certain fluidic components, and installation of the Protasis HTSL-1100 Micro Sample Loader on the right, rear bed of the Gilson. Some rear clearance is needed for the HTSL level with the plane of the white Gilson bed.

To install MicroVAST software, first verify that the Gilson 215 to be used for MicroVAST is working in the VAST environment. Begin on an existing system by issuing the command “loc=1 setvast change” to verify standard operating capabilities, with the syringe size set to 1000 μ L. This should be attempted *without* the Gilson needle in place. An NMR probe need not be connected. The Gilson 215 should execute the setvast macro by retrieving the proper sample and delivering it to the injection port without error. If an error occurs on the Gilson 215 front panel, see the error codes section below.

There are two options for MicroVAST software installation, MicroVAST Installer (an automatic approach), or a manual method. MicroVAST Installer is the recommended method.

MicroVAST Software Installation using the MicroVAST Installer is recommended. See the next section.

MicroVAST Installer

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Introduction

The MicroVAST software is a modification of the Varian VAST flow NMR software that enables use of the Protasis/MRM CapNMR™ probe. MicroVAST Installer saves the original Varian VAST files to a backup location, replaces those files with the modified versions, and installs an application for switching between MicroVAST and the initial version of VAST.

MicroVAST Installer assumes that VAST is already installed and that the TCL interpreter is located at **/vnmr/tcl/bin/nmrtcl**.

Download Installer

Create the **/vnmr/microvast_install** directory.

Download **INSTALL_.TAR** and the **INSTALL_** folder into the **/vnmr/microvast_install** directory.

Expand tar File

From inside the **/vnmr/microvast_install** directory, on the UNIX command line, type:

```
tar -xvf INSTALL_.TAR
```

The **/vnmr/microvast** directory is created.

Operator Interface

- **Remember, Vnmr and VAST must be installed first.**
- Open **microvast.tcl** by typing **microvast.tcl** in a UNIX command shell from the installation directory.
- After initial installation, the configuration program is copied to **/vnmr/microvast/microvast.tcl** and **microvast.tcl** can be opened from there.
- The operator interface is a single check box: labeled **Check to Enable MicroVAST Software** in the TCL/TK script **microvast.tcl**. When checked (colored red) MicroVAST is installed. When un-checked (or not colored) the VAST software is restored to its original state.

Installation

1. Make sure that the unmodified VAST system is already installed.
2. Run the TCL script **microvast.tcl** from a UNIX shell.
3. Specify the installation location; the default is **/vnmr** which is appropriate in most cases.
4. Check the box labeled **Check to Enable MicroVAST Software**.

First Time Install Uses firstinstall Shell Script

Assume **/vnmr** as the local installation directory

1. The installation files are copied to **/vnmr/microvast**. After installation you can run **microvast.tcl** in a UNIX shell from this directory.
2. The original Varian VAST files are copied from their normal locations to the **/vnmr/vast_original** directory so that the original version can be restored as desired.

After First Installation

The **microvast.tcl** script and all supporting files are copied to **/vnmr/microvast**. The configuration utility **microvast.tcl** can be opened from this new location from a UNIX command line.

Checking "Check To Enable MicroVAST Software" Uses the mvinstall Shell Script

1. The MicroVAST files are copied from **/vnmr/microvast** to their proper locations.
2. A time-stamped entry is added to **/vnmr/microvast/microvast.log**
3. The state of the installation (YES) is stored in **/vnmr/microvast/microvast_installed.txt**

Toggling Between MicroVAST and VAST

The MicroVAST Installer described here allows the user to toggle between MicroVAST and VAST. Un-Checking **Check to Enable MicroVAST Software** uses the **mvremove** shell script to restore the original VAST software.

1. The original VAST files are restored from the **/vnmr/original_vast** directory.
2. A time-stamped entry is added to **/vnmr/microvast/microvast.log**
3. The state of the installation (NO) is stored in **/vnmr/microvast/microvast_installed.txt**

MicroVAST Software Installation Notes

General Comments

- 1) Moving files to the `/vnmr/` directory
 - Be logged in as VNMRI
 - `/vnmr` is link to local system home (`cd ~`)
- 2) On a UNIX command line, if you type `tar -xvf installer_file_name`
 - Will display extracted file name and details
 - Check `/vnmr/microvast/` folder for new files

```
cmdtool - /bin/csh
asm          jpsg          par900       tmp
autotest     jre           parlib       tune
bin          lib           probes      user_templates
bootup_message maclib        psq         userlib
conpar       manual        psplib      varian.xicon
conpar.3rf   map          rc.vnmr     vast_original
conpar.start map.tar       satellites   vnmrmenu
devicenames menulib       seqlib      vnmrrev
devicetable microvast.old shapelib    wavelib
dialoglib    microvast_soucre shimmethods xvfonts
ui300:vnmr1 12>cd MicroVAST
ui300:vnmr1 13>pwd
/export/home/vnmr61c/MicroVAST
ui300:vnmr1 14>ls
INSTALL_     INSTALL_.TAR
ui300:vnmr1 15>tar -xvf INSTALL_.TAR
x install_microvast, 0 bytes, 0 tape blocks
x install_microvast/MicroVASTInstaller.html, 4029 bytes, 8 tape blocks
x install_microvast/README, 2914 bytes, 6 tape blocks
x install_microvast/firstinstall, 1391 bytes, 3 tape blocks
x install_microvast/get.tcl, 1 bytes, 1 tape blocks
x install_microvast/gilson, 59851 bytes, 117 tape blocks
x install_microvast/htsl215.gif, 113907 bytes, 223 tape blocks
x install_microvast/inject.tcl, 2632 bytes, 6 tape blocks
x install_microvast/libMicroVAST.tcl, 6075 bytes, 12 tape blocks
x install_microvast/microvast.tcl, 512 bytes, 1 tape blocks
x install_microvast/mrmprobe.gif, 71057 bytes, 139 tape blocks
x install_microvast/mvinstall, 951 bytes, 2 tape blocks
x install_microvast/mvremove, 694 bytes, 2 tape blocks
x install_microvast/put.tcl, 2161 bytes, 5 tape blocks
x install_microvast/retrieve.tcl, 1 bytes, 1 tape blocks
x install_microvast/samp1, 418 bytes, 1 tape blocks
x install_microvast/wash.tcl, 2607 bytes, 6 tape blocks
ui300:vnmr1 16>
```

- 3) On a UNIX command line, if you type `microvast.tcl`
 - This will open new window with probe
 - Clicking and unclicking toggles between MicroVAST or VAST

Check for:

- `/vnmr/asm/tcl/` – directory for `.tcl` files rewritten for today's date
- `/vnmr/bin/gilalign` – important Gilson file
- `/vnmr/tcl/bin/gilson` – important Gilson file and primary gilson macro

4) Vnmr Commands for Gilson Macro

- vrack** - sample rack
- loc=1** - sample location at well 1
- loc='n' will disables MicroVAST from picking up a sample
- don't run same **loc** value twice in a row (within a specified well plate)

5) Vnmr Command for Multiple Runs

- enter** - macro that sets up samples (will query for a automation name)
- autogo** - macro that starts multiple run (will query for a data directory)

6) Helpful UNIX Tools & Commands

- cd ~** - in command tool, will display system home directory
- textedit &** - opens a blank text editor
- more file_name** - displays text for file **file_name**
- filemgr &** - opens file manager in background (allows click and drag)
- snapshot &** - opens utility for a screenshot (only on Sun & Solaris)
- tar -xvf file_name** - extracts a tar file (with verbose text)
- cannot have additional compression like a **.tar.gz** file

Manual MicroVAST Software Installation (Usually Not Recommended)

Once the Gilson 215 runs the above VAST command (or similar) without error, VAST software will need to be modified on the spectrometer workstation. Replace the five VAST tcl scripts within /vnmr/asm/tcl/filename. The suffix .tcl is added by the system, so drop the .tcl before copying into place. The script files are:

- get.tcl (a required blank file)
- retrieve.tcl (a required blank file)
- inject.tcl
- put.tcl
- wash.tcl

Do not overwrite your existing tcl code or any other VAST program. Instead, move the standard file to a recorded location, or rename it in an obvious or otherwise recorded manner. To edit tcl code, do so as vnmr1.

Also, replace the VAST file named gilson, by placing the new gilson file in /vnmr/tcl/bin/filename. Be sure to make the file named gilson executable. Again, be sure to move the standard file to a recorded location, or rename it in an obvious or otherwise recorded manner.

Testing MicroVAST

Test the system by typing in the command:

```
loc=1 go (will acquire NMR data)
```

or

```
loc=1 change (will not acquire NMR data)
```

Keep in mind that if you repeat this command, you cannot use the same loc value twice in a row.

If the system does not function properly at this point (and no Gilson 215 error is indicated), update your versions of Roboproc and gilalign from the Varian website. This can be accomplished by updating your version of vnmr6.1c, including patches. Updating version vnmrj1.1c is also acceptable.

- The program gilalign is placed in /vnmr/bin/filename.
- The program Roboproc is placed in /vnmr/acqbin/filename.

Permissions can cause problems. If these arise, try this:

- backup the asm directory by copying it entirely to some chosen backup name
- as root, type “chown -R vnmr1 asm; chmod -R 777 asm”
- retry gilson command

An “su acqproc” command is necessary at times to clear certain errors.

If gilalign is actually run, run it only from the command line. However, one would not typically need to run the gilalign program separately.

For successive, automated runs, type

```
enter
```

on the Vnmr command line. This opens a 96-well panel that is used in the same manner as with VAST. Type

```
autogo
```

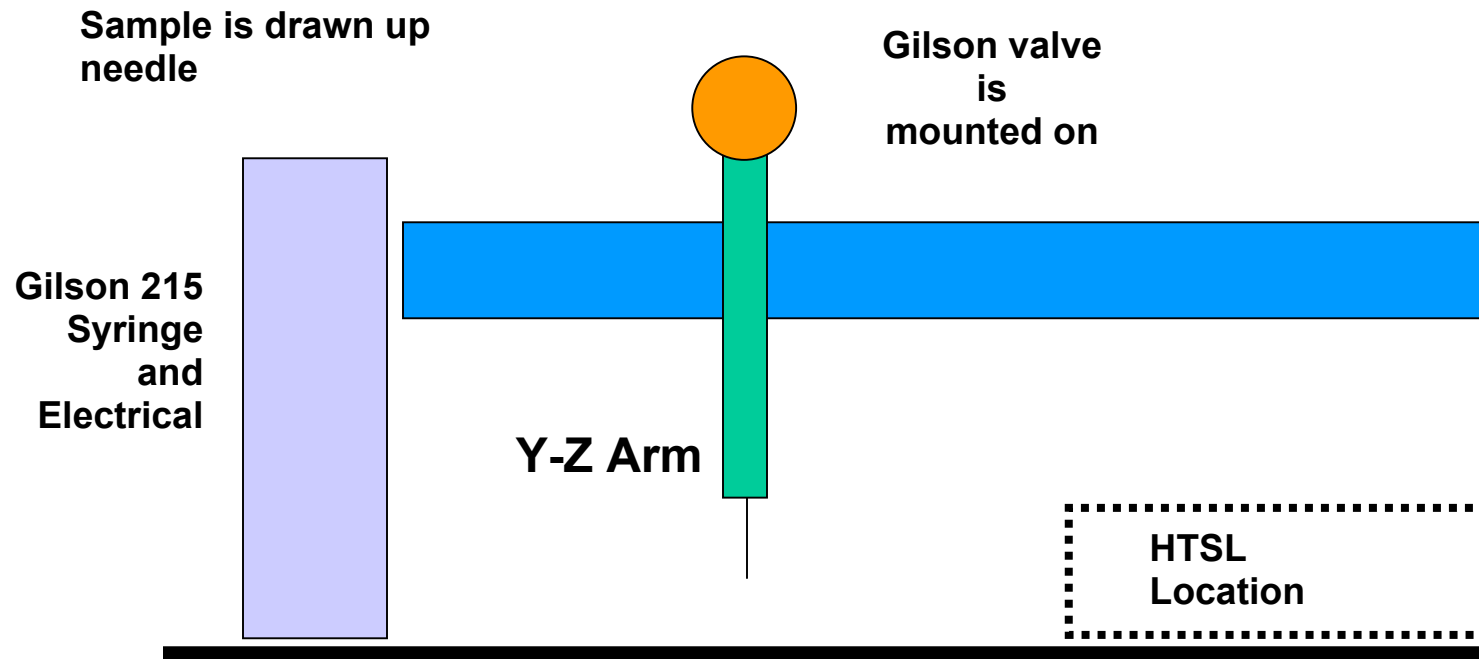
to run the samples declared in the enter window, just like with VAST.

Hardware Conversion from Conventional VAST to MicroVAST

- Remove the plumbing (tubing and fittings) from the Gilson 215.
- The solvent uptake tubing connected to the syringe valve can be retained if desired.
- Remove the Gilson needle.
- Remove the Gilson syringe (move the syringe plunger to the lowered position first).
- Remove the Gilson valve and housing (usually model 819), and bracket which sits at the right rear.
- Remove any gas control apparatus if it interferes with MicroVAST operation.
- Proceed with the installation of MicroVAST

See Diagram on the next page

VAST to MicroVAST Conversion: Z-Arm Valve Configuration



VAST to MicroVAST conversion:

- Install HTSL on Gilson bed (Once at initial install)
- Bypass Gilson valve
- Modify VAST software to MicroVAST

Gilson 215 Operational Notes

Firmware. MicroVAST will run on Gilson 215 firmware versions as low as 1.21. You can determine the firmware version by powering on the Gilson and watching its front panel. It briefly flashes the firmware version right after powering on. You might need a new electrical assembly board if a certain error occurs (see below). The firmware version on it will be 2.50 (as of November 2003).

Z-Arm. If you begin with a Gilson fitted with a 175 mm z-arm, you will need to change the z-arm to the 125-mm size to accommodate the Gilson microneedle. Neither VAST nor MicroVAST accommodate the 175-mm z-arm, which you can trade-in or replace. The 125-mm z-arm part number is #250610 and is ordered from Gilson at 1-800-445-7661 (ask for Dusty). Replacement cost is \$796.00, and direct purchase cost is \$1232.00 as of November 2003. Upon installing the 125-mm z-arm, you need to specify the 125-mm z-arm size for that particular Gilson 215 by using the Gilson Utilities software.

Gilson Errors. Errors 13 and 14 are the most common errors to occur. Error 13 is “Syringe speed out of range” and Error 14 is “Invalid syringe volume”. Neither error indicates a hardware failure, but instead an incompatibility of conditions, usually between the specified sample volume and the VAST command sequence with parameters set by a macro or in the gilson parameters window. Changing to compatible conditions usually solves the problem. To recover from either error, press the Start button on the front of the Gilson. You may also need to close and re-open the gilson window (reconnect), and/or perform an su acqproc sequence.

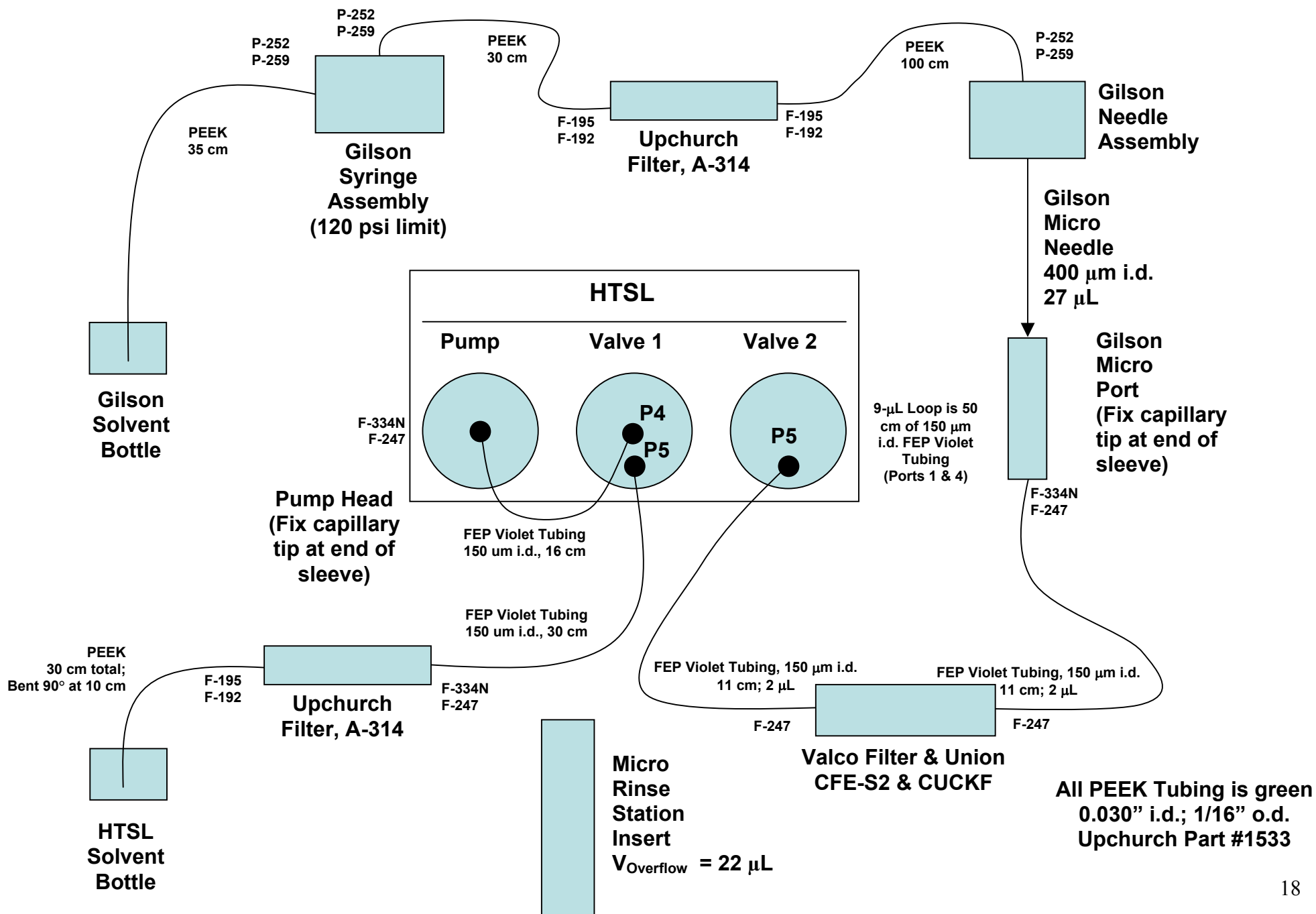
Error 33, 34, or 35 indicate at the x, y, or z home phase is invalid, and this can occur upon power-up. Running the home phase procedure in set_215.exe or 215setup.exe in your Gilson Utilities software will usually solve the problem. If this does not clear the error, and the Gilson 215 still fails to home upon power-up, you may need a new electrical assembly board at a cost of about \$300.00 (November 2003). Call MRM or Gilson for advice on this situation.

We recommend that the conversion to MicroVAST begin with a Gilson 215 that has been used successfully for VAST before. If this is not the case, several problems can occur. The 125-mm z-arm size is required, and the size must be declared via the Gilson Utilities software. Also, the Gilson may need to have its default conditions restored. Two types of commands, Immediate and Buffered, can be executed in the Gsutil32.exe program in the Gilson Utilities software, as listed in the Gilson 215 Liquid Handler User’s Guide.

Other Gilson errors and their remedies are listed in the Gilson 215 Liquid Handler User’s Guide.

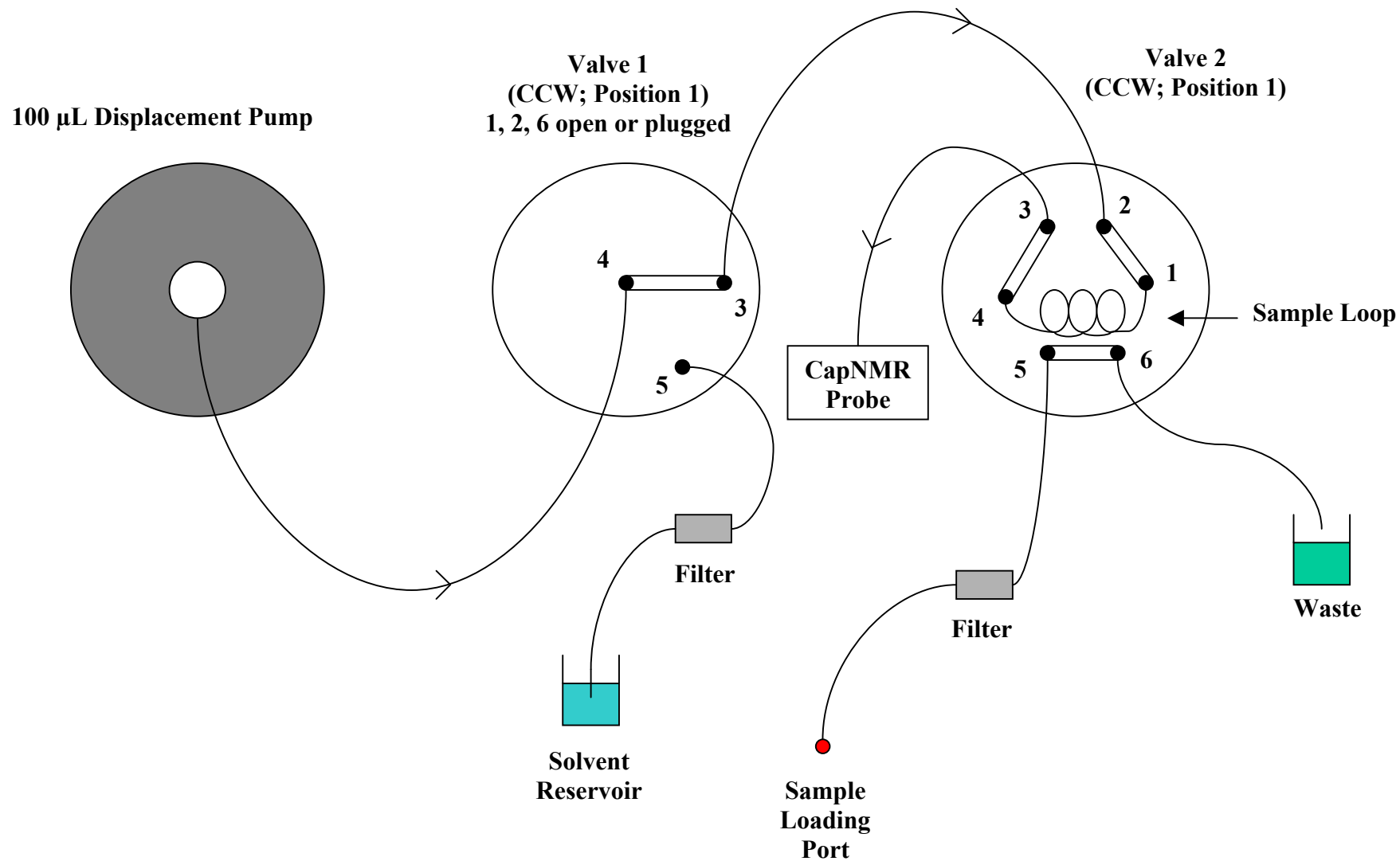
Rotary Switches. On the rear, right side of the Gilson 215, two rotary switches are located labeled SW1 and SW2. We have ours both set to 2. SW1 sets the Gilson 215 i.d. to 22, and SW2 sets the baud rate to 19,200. SW1 is probably already set to 2 on your unit, which makes the unit i.d. 22. You will probably not need to touch SW2 even if it is not set to 2, as it is probably not used in MicroVAST.

MicroVAST Layout: Gilson, HTSL, and FEP 1/32" Tubing

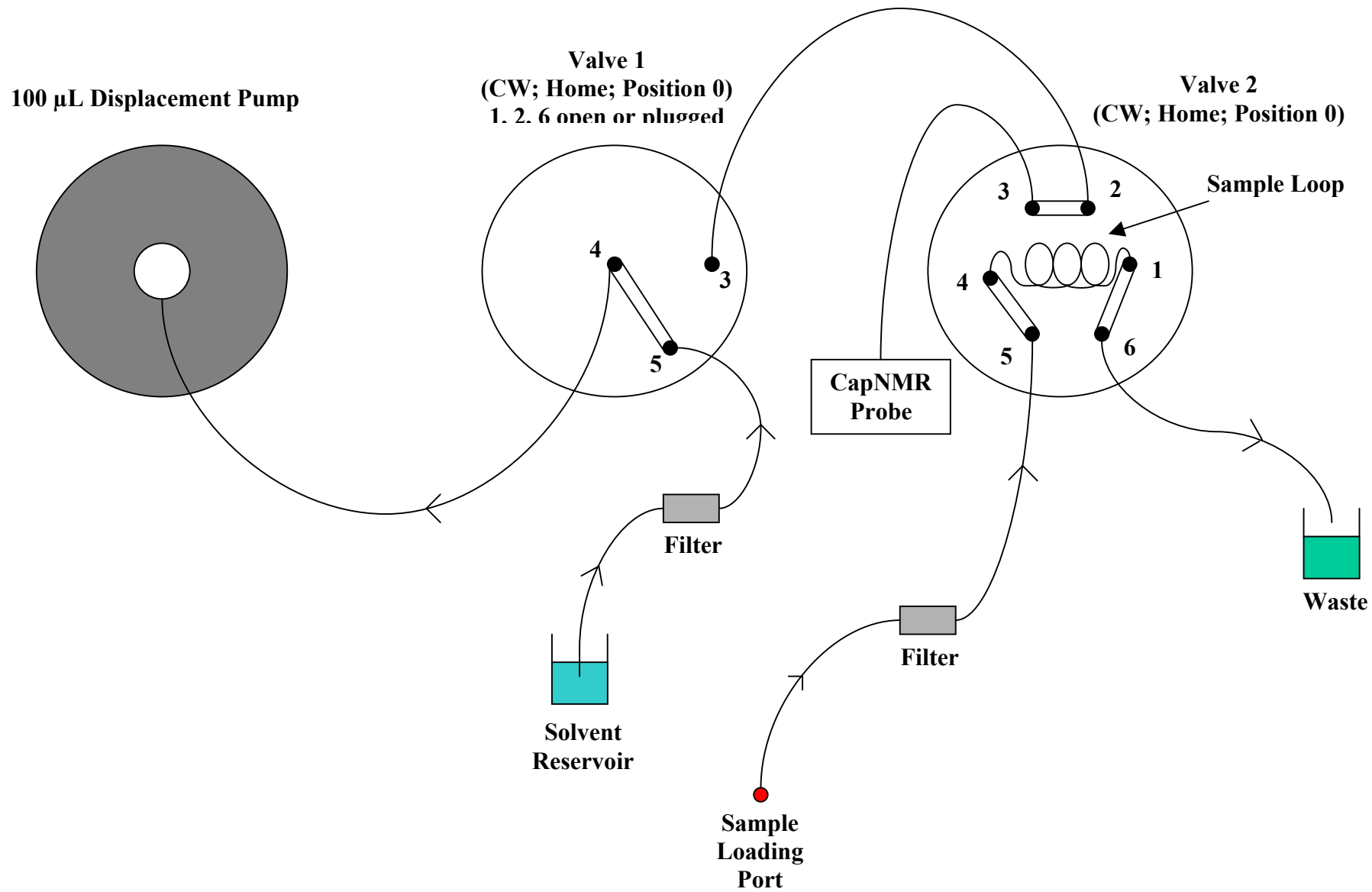


HTSL Fluidic Valve Diagram FEP Version

Status: HTSL Deliver



HTSL Fluidic Valve Diagram FEP Version
Status: HTSL Refill / Load Loop / Sample Parked

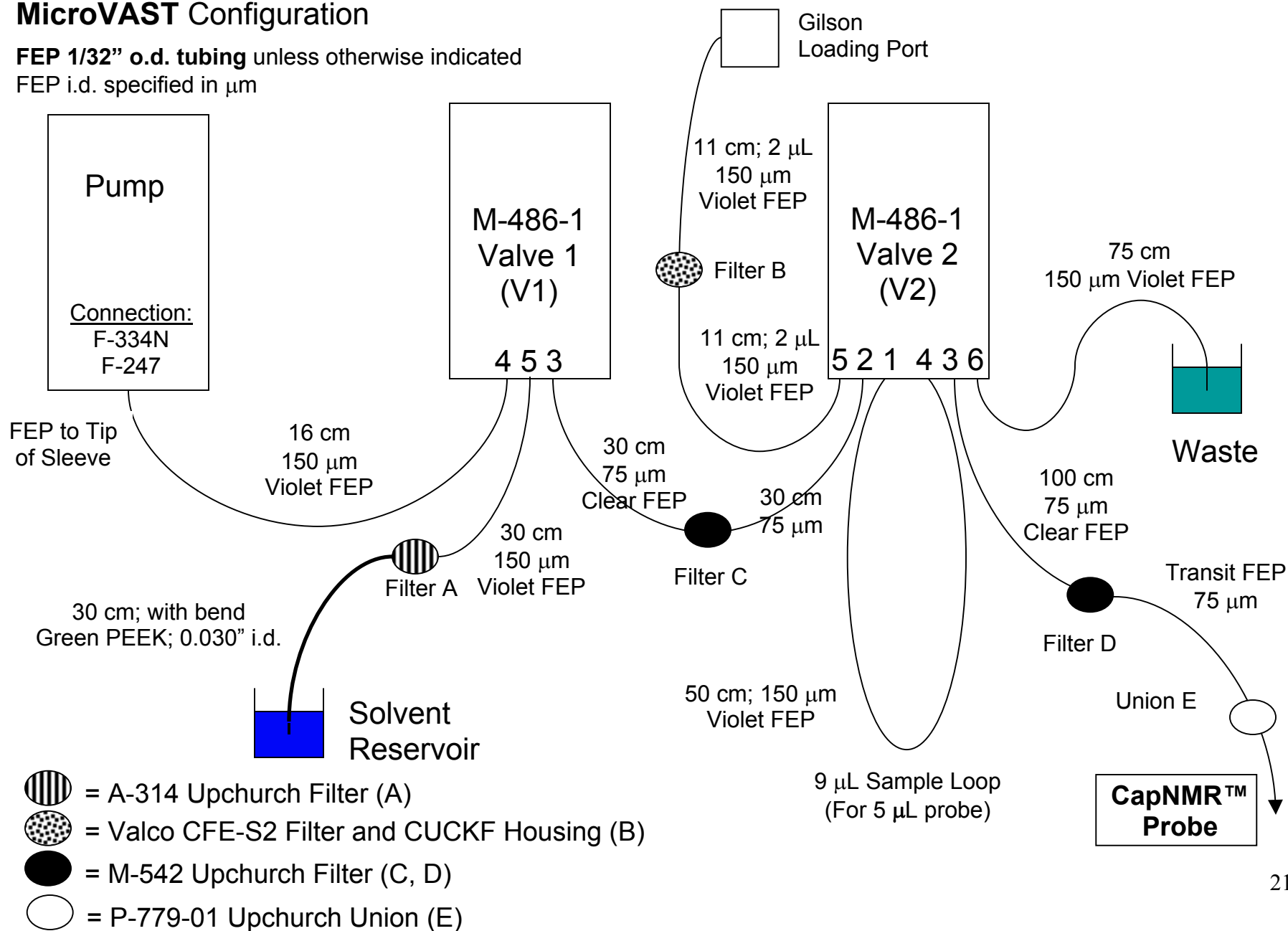


HTSL-1100 Fluidic Layout

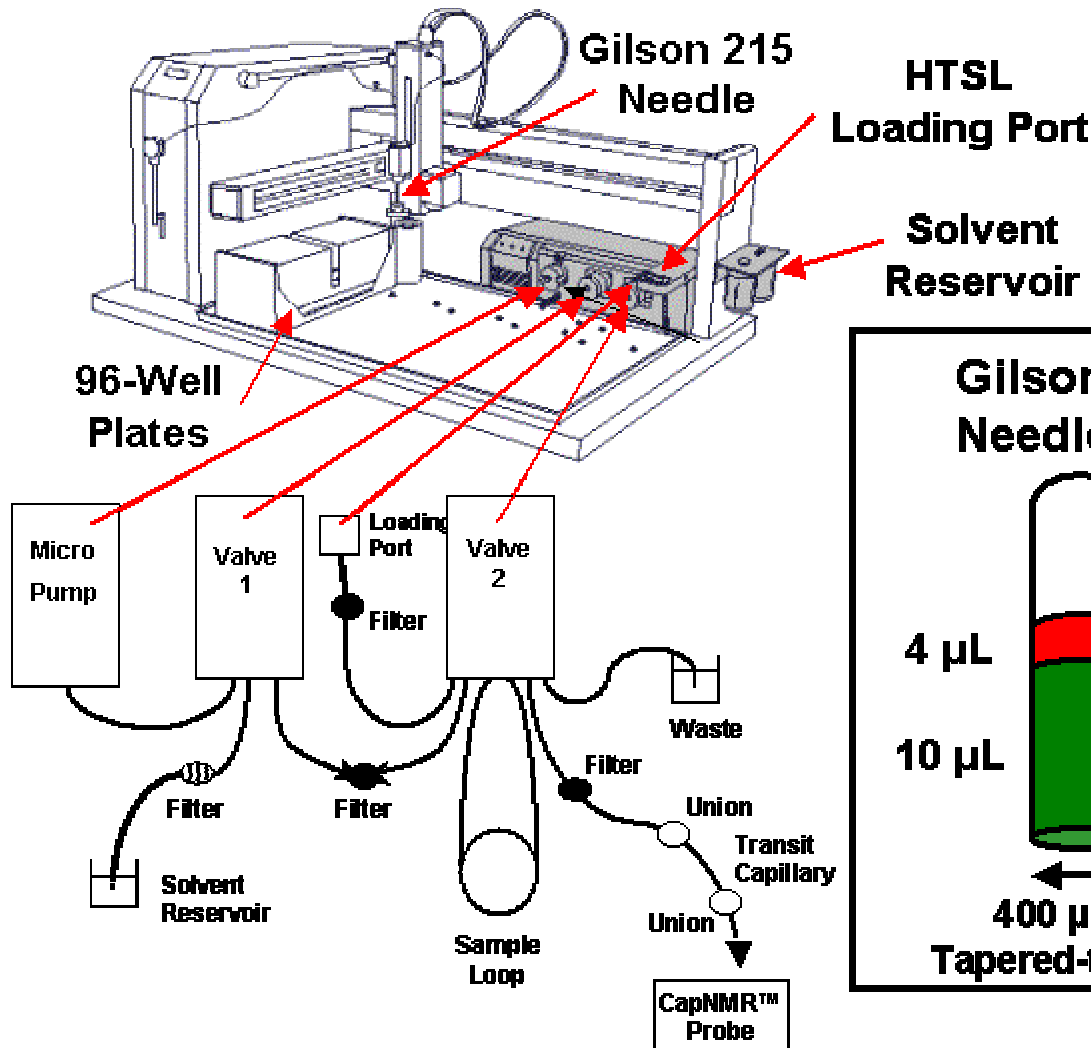
MicroVAST Configuration

FEP 1/32" o.d. tubing unless otherwise indicated

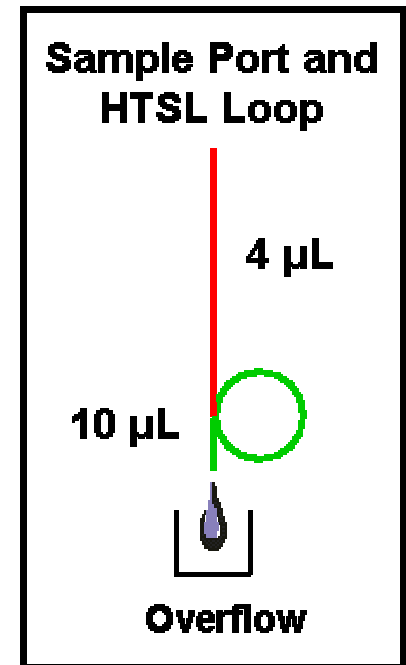
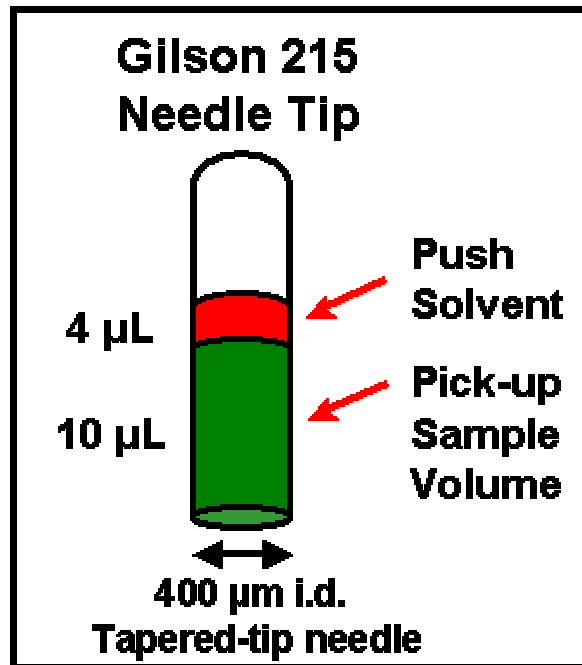
FEP i.d. specified in μm



Automated Flow Injection: MicroVAST Basic Operation



- Push solvent: 4 μL
- Pick-up volume: 10 μL
- Load volume: 14 μL
- Inject volume: 9 μL
- Loop volume: 9 μL



Troubleshooting

If an error is encountered in operation, it usually appears on the Gilson 215 panel with an error number. Errors often occur during system initialization, or when a switch is made from one Gilson 215 to another.

An “su acqproc” command is necessary at times to clear certain errors. Here is a description of the two options that invoke this UNIX command. The su acqproc command is run through a UNIX command tool window.

1) The su acqproc command sequence alone:

- Open the window and type “su acqproc”, and the response will be “killing acqproc”
- Wait about 30 sec
- Type “su acqproc” again, and the response should be “starting acqproc”

2) Reboot of the console, including su acqproc sequence:

- Open the window and type “su acqproc”, and the response will be “killing acqproc”
- Wait about 30 sec
- Reboot the console
- Type “su acqproc” again, and the response should be “starting acqproc”

If you encounter an error that the above actions do not correct, be sure the settings in the Varian VAST enter window are appropriate for syringe size you have declared, which for MicroVAST is 100 μ L.

Contact MRM directly if any questions arise.

Gilson/HTSL Cable Connection Scheme

Here is the cable connection scheme with which we have operated our Gilson 215 and HTSL using the Gilson software. The cable comes pre-assembled.

8-Terminal Connector to the Autosampler (Gilson Input; HTSL Output)

8-terminal connector, numbered 1 to 8, top to bottom, looking at screws, wires departing to the left on green terminals, or wires departing to the right on black terminals. The key is that the round-ribbed portion of the terminal needs to point to the left (while looking at the screws) to get the orientation correct with respect to the Gilson 215.

Terminal 1 - solid brown wire (= pin 8 on HTSL clip connector)

Terminal 2 - striped brown wire (= pin 7 on HTSL clip connector)

These two wires are joined by a contact closure event created by the HTSL.

10-Terminal Connector to the Autosampler (Gilson Output; HTSL Input)

10-terminal connector, numbered 1 to 10, top to bottom, looking at screws, wires departing to the left on green terminals, or wires departing to the right on black terminals. The key is that the round-ribbed portion of the terminal needs to point to the left (while looking at the screws) to get the orientation correct with respect to the Gilson 215.

Terminals 1 and 7 - jumpered by a separate wire, any color you want

Terminal 2 - striped orange wire (= pin 1 on HTSL clip connector; ground)

Terminal 8 - solid orange wire (= pin 2 on HTSL clip connector; +24 VDC from Gilson)

These two wires transmit a contact closure event created by the Gilson 215 with +24 VDC on terminal 8.

Parts

Cable, Belkin Fastcat 5e, #R7J604, with standard ethernet, 8-pin, clip-type connector goes into HTSL

8-Terminal green connector, Gilson part #638308512; \$14.00 each

10-Terminal green connector, Gilson part #638310512; \$21.00 each

TCL Code and the Varian gilson File

These files should be obtained directly from MRM personnel.

get.tcl

This file is blank, but must be included.

retrieve.tcl

This file is blank, but must be included.

inject.tcl

See text below.

put.tcl

See text below.

wash.tcl

See text below.

gilson

This file is compiled, and not listed here as text.

inject.tcl

```
# @(#)inject.tcl 5.1 12/08/98 Copyright (c) 1996-1997 Varian Assoc.,Inc. All Rights Reserved
#
# Varian Assoc.,Inc. All Rights Reserved.
# This software contains proprietary and confidential
# information of Varian Assoc., Inc. and its contributors.
# Use, disclosure and reproduction is prohibited without
# prior consent.
#
# Edited by Patrick Wheeler, 09/25/2002
# Edited by MRM 6Aug2003 ,7 Aug
# added pushvolume to Aspirate Sample
# added DelayHTSLcycle in place of HTSL trigger

proc inject { RackLoc SampleNumber SampleZone SampleVolume SampleHeight SampleDepth } {

    global ProbeVolume MaxFlow SampleWellRate SampleZSpeed ProbeFastRate DelayHTSLcycle

    gPuts "----- MicroInject.tcl-----\n"

    # ---- Start of putSample Script -----

        set debug 1
        set msg [format "R%dZ%dS%d" $RackLoc $SampleZone $SampleNumber ]
        gWriteDisplay "$msg"

    # ----- Pick up the needle -----
    gPuts "Lift needle up \n"

        gMoveZ2Top

    # ----- Move to the Sample Location -----
    gPuts " Moving to rack= $RackLoc Zone= $SampleZone Sample= $SampleNumber \n"
        gMove2Sample $RackLoc $SampleZone $SampleNumber

    # ----- Put Needle into liquid -----
    gPuts " Lowering Needle into Sample \n"
        gMove2LiqLevel $RackLoc $SampleZone $SampleNumber $SampleHeight $SampleDepth

    # ----- Aspirate Sample -----
        set msg [format "Z%dS%dAS" $SampleZone $SampleNumber ]
        gWriteDisplay "$msg"

    gPuts " Pulling up $ProbeVolume \n"
        set PushVolume [expr ($ProbeVolume - $SampleVolume)]
        if {$PushVolume < 0.0} { set PushVolume 0.0 }

        if {$PushVolume > 0.0 } {
            gFlush $PushVolume $SampleWellRate 0
        }
        gAspirate $SampleVolume $SampleWellRate $SampleZSpeed
        gDelayMsec 2500

    gPuts "Lift Needle up and move to Injector Port \n"
        gMoveZ2Top
        gMove2InjectorPort

    # ----- Inject Sample + Push Volume == ProbeVolume -----
```

```

gPuts "Inject Sample plus any push solvent into HTSL1100 Loop \n"
  gDispense $ProbeVolume $ProbeFastRate 0
  set msg [format "R%dZ%dS%d" $RackLoc $SampleZone $SampleNumber ]
  gWriteDisplay "$msg"

gPuts "Signal HTSL-1100 to start \n"

gDelayMsec 8000
gSetContacts 3 1
gDelayMsec 5000
gSetContacts 3 0

gDelayMsec $DelayHTSLcycle

#-----wait for HTSL trigger-----

# set conval [gGetInputs 1 ]

# gPuts "Input: 1 = $conval\n"

# while { $conval != 1 } {
#   gDelayMsec 1000
#   set conval [gGetInputs 1 ]
#   gPuts "Contact 1 = $conval\n"
# }

# gPuts "HTSL-1100 Pump has parked the sample \n"
# gSetContacts 3 0

gPuts " End of MicroInject Protocol \n"
}

```

put.tcl

```
# @(#)put.tcl 5.2 09/15/99 Copyright (c) 1996-1997 Varian Assoc., Inc. All Rights Reserved
#
# Varian Assoc., Inc. All Rights Reserved.
# This software contains proprietary and confidential
# information of Varian Assoc., Inc. and its contributors.
# Use, disclosure and reproduction is prohibited without
# prior consent.
#
# Modified by PDW for 2-valve system, 29 Jan 2002
# Checked for compatibility with MRM probe & HTSL-1100, 3 Oct 2002
#

gPuts "----- put.tcl-----\n"
set not_injected_yet 1

source $env(vnmrsystem)/asm/tcl/wash.tcl
source $env(vnmrsystem)/asm/tcl/inject.tcl
source $env(vnmrsystem)/asm/info/racks
source $env(vnmrsystem)/asm/info/default
catch { source $env(vnmrsystem)/asm/info/sampInfo }

gPuts "----- put.tcl: source $SampleInfoFile\n"

if {[catch {source $SampleInfoFile}] == 1} {
    if {[catch {source $env(vnmrsystem)/asm/info/current}] == 1} {
        gPuts "----- put.tcl: No Current, Skipping put.tcl\n"
        return 0
    }
}

# ----- message to gilson's display -----
set msg [format "R%dZ%dS%d" $RackLoc $SampleZone $SampleNumber ]
gWriteDisplay "$msg"

gPuts "----- put.tcl: source $putProtocol \n"

if ($not_injected_yet) { # putprotocol could have already injected

    set msg [format "R%dZ%dS%d" $RackLoc $SampleZone $SampleNumber ]
    gWriteDisplay "$msg"

    gPuts "calling inject $RackLoc $SampleNumber $SampleZone $SampleVolume $SampleHeight
$SampleDepth\n"
    inject $RackLoc $SampleNumber $SampleZone $SampleVolume $SampleHeight $SampleDepth

# Start acquisition during needle & injector port rinse
ResumeAcq

# Includes wash of injection loop

gPuts " ----- calling wash probe \n"
wash probe

gPuts " ----- calling wash needle \n"
wash needle

set not_injected_yet 0
}
```

```
if { [file exist $SampleInfoFile] == 1} {  
  exec rm -f -f $env(vnmrsystem)/asm/info/current  
  # copy sample in magnet into current  
  exec mv $SampleInfoFile $env(vnmrsystem)/asm/info/current  
  # copy sample flows and volumes of sample in magnet into current  
  exec cat $env(vnmrsystem)/asm/info/sampInfo >> $env(vnmrsystem)/asm/info/current  
}
```

wash.tcl

```
# @(#)wash.tcl 5.1 12/08/98 Copyright (c) 1996-1997 Varian Assoc., Inc. All Rights Reserved
# Varian Assoc., Inc. All Rights Reserved.
# This software contains proprietary and confidential
# information of Varian Assoc., Inc. and its contributors.
# Use, disclosure and reproduction is prohibited without
# prior consent.
#
# Edited by Patrick Wheeler, 3 Oct 2002 for use with HTSL-1100
# Edited by Randy Rutkowske, 29 Sep 2003 to add drawback rinse
#
# ----- Routine to Rinse Needle -----
proc RinseNeedle { } {
    global NeedleRinseVolume NeedleRinseRate NumRinses

    for { set x 1 } { $x <= $NumRinses } { incr x } {

        gMoveZ2Top
        set debug 1

        gPuts "Needle Rinse Uptake. $NeedleRinseVolume $NeedleRinseRate \n"

        gFlush $NeedleRinseVolume $NeedleRinseRate 0.0

        # Move to Rinse Station while syringe is working
        gMove2RinseStation

        gPuts "Needle Rinse Flush. $NeedleRinseVolume $NeedleRinseRate \n"

        gFlush $NeedleRinseVolume 0.0 $NeedleRinseRate

        gMoveZ2Top
    }
}

# =====

# ----- Routine to Rinse and Backflush HTSL-1100 Sample Loop -----
proc RinseNmrProbe { } {

    global ProbeVolume RinseDeltaVol NumRinses MaxFlow ProbeFastRate ProbeSlowVol
    ProbeSlowRate RinseExtraVol NeedleRinseVolume NeedleRinseRate

    set RinseVol [expr 0 + $RinseExtraVol]
    set debug 1
    #    gMoveZ2Top

    for { set x 1 } { $x <= $NumRinses } { incr x } {

        gPuts "Injport rinse uptake. $RinseVol $NeedleRinseRate \n"
        gFlush $RinseVol $NeedleRinseRate 0.0
        gMove2InjectorPort

        gPuts "Injport rinse flush. $RinseVol $ProbeFastRate \n"
        gFlush $RinseVol 0.0 $ProbeFastRate

        gPuts "Backflushing loop. $RinseVol $ProbeFastRate \n"
```

```

gAspirate $RinseVol $ProbeFastRate 0.0
gDelayMsec 1000
gMoveZ2Top
gMove2RinseStation
gDispense $RinseVol $ProbeFastRate 0.0
gMoveZ2Top

set vol $ProbeSlowVol
if {$vol > ($RinseVol + $RinseExtraVol)} {
    set vol [expr $RinseVol + $RinseExtraVol]
}

}

}
# =====

proc wash { who } {

    global SampleNumber SampleZone

    if { $who == "needle" } {

# ----- Rinse Needle, Gilson calls this the probe -----
set msg [format "Z%dS%dRN" $SampleZone $SampleNumber ]
gWriteDisplay "$msg"
RinseNeedle

    } elseif { $who == "probe" } {
# ----- Rinse the NMR Probe -----
set msg [format "Z%dS%dRP" $SampleZone $SampleNumber ]
gWriteDisplay "$msg"
RinseNmrProbe

    } else {
        error "Usage: wash [needle | probe | injport]"
    }
}

```

End of Document