BRUKER

Bruker BioSpin

BNMI-HP

Bruker NMR-MS Interface, High Performance

User Manual - Version 002

think forward

NMR Solutions

The information in this manual may be altered without notice.

This manual was written by

David Kilgour

Rheinstetten, Germany

P/N: Z31813
DWG-No.: Z4D10302 - 002

CE

DECLARATION OF CONFORMITY

The undermentioned product

BNMI-HP (LC-NMR-MS Interface) H10193

conforms to the main requirements
set by the commission for the
Harmonization of Regulations of the EU Member States
with regards to electromagnetic compatibility
(EMI 89/336/ECC) and safety (Low Voltage Electrical
Equipment: 72/23/ECC) regulations.

For the assessment the following norms were applied:

EMI: EN 61326-1: 2001
Test report: Nemko FS-0606-69357
Safety: EN 61010-1: 2nd ed. (2001)
Test report: INNOVA Product Service GmbH 06KFS085

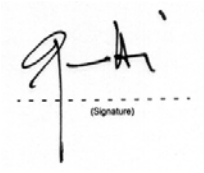
Manufacturer's Name: BRUKER BIOSPIN GmbH
Manufacturer's Address: 76287 Rheinstetten, Silberstreifen, Germany

Declaration approved by:

Dr. Tonio Gianotti

Head of Development

Rheinstetten 09.10.2006



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Introduction 1.1

This manual is included in the delivery of the BNMI-HP.
It instructs how to:

- install and configure the BNMI-HP unit
- operate the unit
- perform basic fault finding on the unit

Disclaimer 1.2

The unit should only be used for its intended purpose as described in this manual. Use of the unit for any purpose other than that for which it is intended is taken only at the users own risk and invalidates any and all manufacturer warranties.

Service or maintenance work on the unit must be carried out by qualified personnel.

Only those persons schooled in the operation of the BNMI-HP should operate the unit.

Read this manual before operating the unit. Pay particular attention to any safety related information.

Safety Issues 1.3

The unit is intended for use in conjunction with HPLC, NMR and MS systems. As such, standard solvents used in these systems may be pumped through the BNMI-HP system under high pressure. Only personnel schooled in the use and dangers of these solvents should work directly with the unit. (Refer to 'Good Laboratory Practice' regarding handling of solvents)

Warnings and Notes 1.4

There are two types of information notices used in this manual. These notices highlight important information or warn the user of a potentially dangerous situation. The following notices will have the same level of importance throughout this manual.

Note: Indicates important information or helpful hints

WARNING: Indicates the possibility of severe personal injury, loss of life or equipment damage if the instructions are not followed.

Contact for Additional Technical Assistance 1.5

For further technical assistance on the BNMI-HP unit, please do not hesitate to contact your nearest BRUKER dealer or contact us directly at:

BRUKER BioSpin GMBH
am Silberstreifen
D-76287 Rheinstetten
Germany

Phone: + 49 721 5161 0
FAX: + 49 721 5171 01
Email: lcnmr@bruker.de
Internet: www.bruker.de

Terms and Definitions

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• NMR	Nuclear Magnetic Resonance
• MS	Mass Spectroscopy
• HPLC	High Performance Liquid Chromatography
• BPSU36	Bruker Peak Sampling Unit used to store up to 36 HPLC separations for later analysis in an NMR and/or an MS System.
• BPSU36-2	Successor to the Bruker Peak Sampling Unit used to store up to 36 HPLC separations for later analysis in an NMR and/or an MS System. Uses High pressure valves, has a 100Mbit Ethernet Interface and operates with an 8Mb Storage Cassette.
• BNMI	Bruker NMR-MS Interface
• BNMI-HP	Bruker NMR-MS Interface, High Performance.
• BSFU-O	Bruker Stopped Flow Unit (Oven). Used in conjunction with an HPLC Pump and the BPSU36 to perform HPLC.
• BMSO	Bruker Multi Column Switching Oven: Successor to the BSFU-O Operates together with the BPSU36-2 or in 'Stand-Alone' mode.
• Esquire	Bruker Ion Trap MS System
• Esquire 3000	Successor to the Esquire
• LC-NMR	Combined HPLC and NMR analysis
• LC-NMR-MS	Combined HPLC, NMR and MS analysis
• HyStar	Bruker PC (Windows 2000, XP) Program integrating control of the various HPLC systems + BPSU36(-2), BSFU, BNMI(-HP) and Bruker MS systems.
• UV	Ultra Violet
• Peak	A peak is an HPLC separation which has been identified either as a UV absorptions peak or as an MS Peak.
• FTP	File Transfer Protocol (used for download)
• TFTP	Trivial File Transfer Protocol (used by bootloader)
• DHCP	Dynamic Host Configuration Protocol
• IP	Internet Protocol

Terms and Definitions

LC-NMR-MS Interface

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BNMI-HP Delivery Contents 3.1

- **BNMI-HP unit**
- **Mains connector cable and 2m network cable**
- **2 x 250ml Flasks with standard and through-hole caps**
- **Accessory kit with tubing, filters, spare fittings and fuses, etc.**

BNMI-HP Basics 3.2

The LC-NMR-MS Interface is designed to sit between an HPLC system (probably including a BPSU36-2 and BMSO), an NMR spectroscopy probehead and an MS unit. It would normally be mounted on top of the MS table immediately in front of the MS input connector. *The unit must not be operated while sitting on a soft surface. The air intake vents are on the base of the unit and these must not be blocked.*

There are 2 versions of the BNMI-HP:

H10193 Variant 0: without pump for calibration fluid

H10193 Variant 1: with pump for calibration fluid

- **Any commands, operations or status information with respect to the calibration pump apply only to Variant. 1**

The BNMI-HP consists of 2 (or 3) medium pressure (> 60Bar) syringe pumps, a 1:20 splitter, an 8-Port valve, 3 mixing chambers (T-Pieces) and associated control and interface electronics. The flow of the HPLC separations to the MS unit can be modified by pumping additional solvent from the syringe pumps. This improves the MS peak detection and shortens the time needed for the split separation to reach the MS. Separations can also be stored as a result of an MS peak detection as well as through UV absorption peak detection.

The splitter splits the fluid flow from the HPLC in a 1:20 ratio. i.e. ca. 5% of the fluid is directed to the MS and the remainder to either the NMR probehead for immediate analysis or to the BPSU36 storage cassette for storage and later analysis.

The fluid directed to the MS is mixed in the mixing chamber (T-Piece) with the solvent pumped from the syringe pumps. The two left hand syringe pumps pump alternatively to guarantee a constant 'makeup' flow. The 3rd pump at the right hand side is used to pump a calibration fluid into the flow path to allow the MS to be calibrated (or simply synchronized) at the start and end of each measurement cycle.

Continued after the next 2 pages, after the diagrams.

Figure 3.1. BNMI-HP Tubing Connections Layout

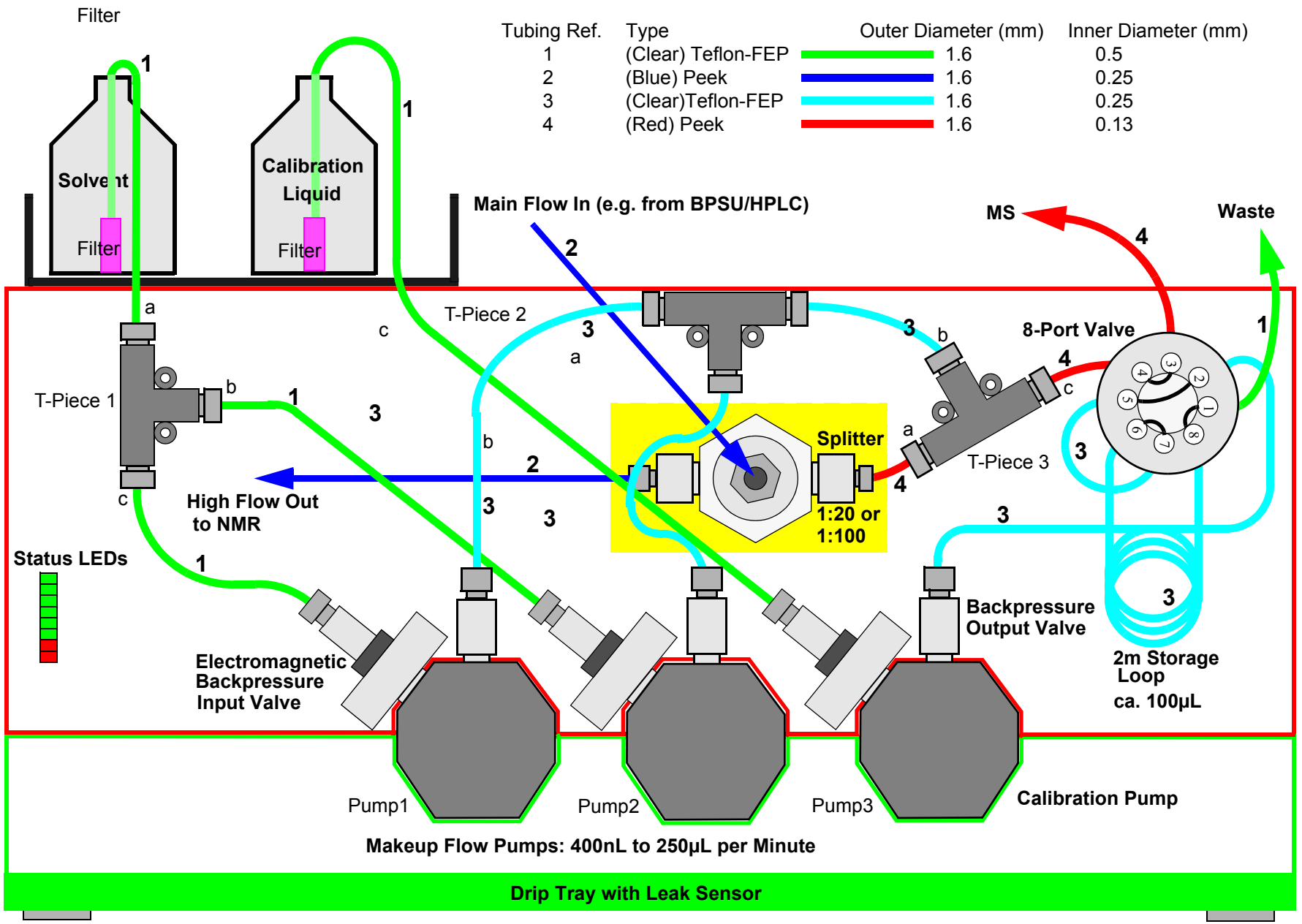
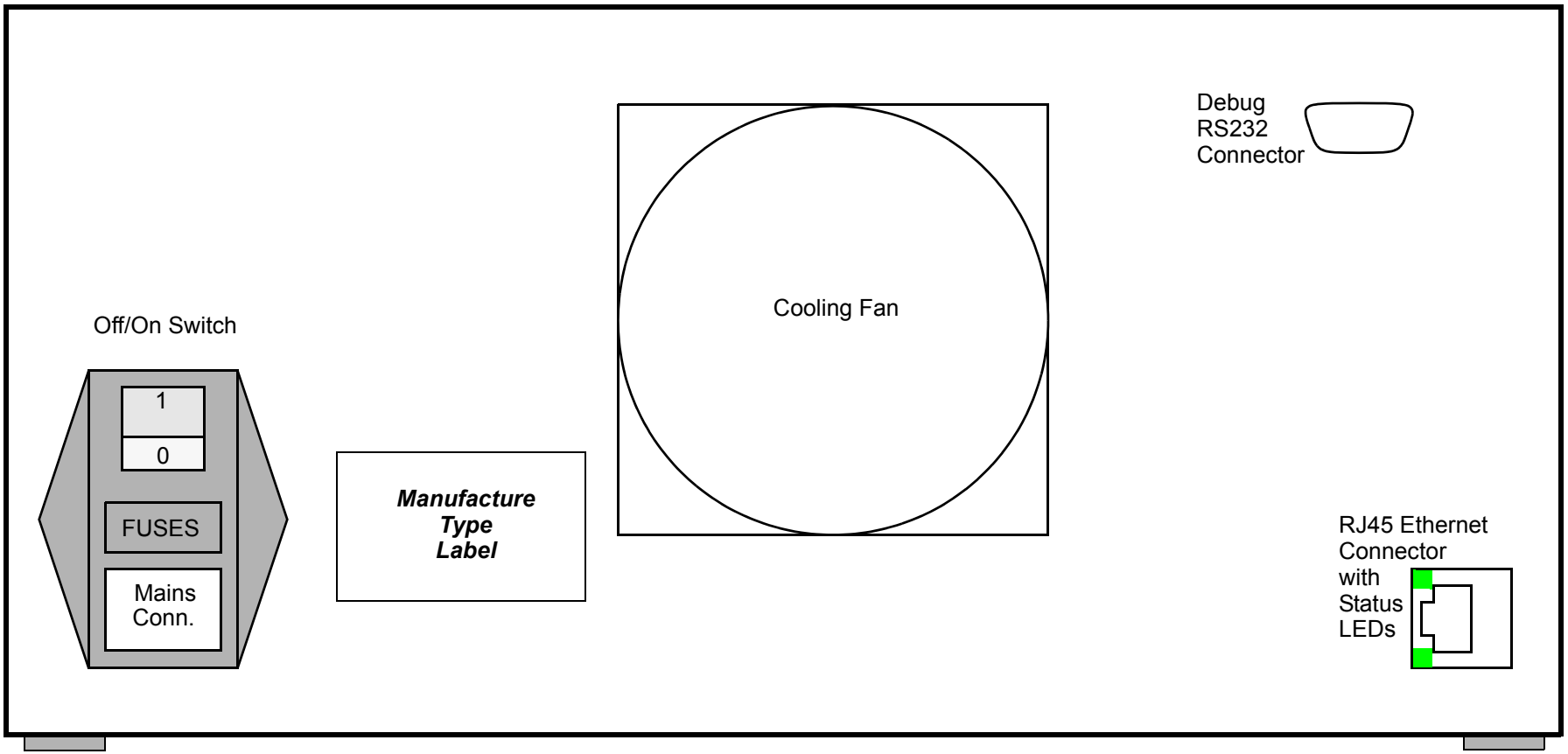


Figure 3.2. BMNI-HP Rear View showing Electrical Connections



Default RS232 Configuration: 19200 Baud, 8 Data Bits, 2 Stop Bits, No Parity.
Default Ethernet: 100MBit, IP 192.168.254.42
The IP address can be changed via the debug interface.
See ***"The Debug Interface" on page 58***

The 8-Port valve is used to direct the fluid flow. Control of the BNMI Unit is performed using the HyStar program via an ethernet interface. Commands received from HyStar are interpreted in the BNMI Control Board. The control board then sets up the BNMI unit accordingly e.g.: Starts the pumps, changes the flow rate, turns the 8-Port valve. The splitter has a fixed mechanical function and cannot be controlled or adjusted. It simply accepts a fluid flow and splits it into 2 paths in a 1:20 ratio. However, other split ratios are available (for example 1:100 may be preferred if you have very high sample concentrations).

Installing The BNMI-HP *3.4*

The BNMI-HP unit should be mounted on the Pump Table in front of the MS unit or if using an older Esquire MS, the unit can sit on top of the MS. Ensure it is located such that the path from the 8-Port valve to the MS-Input connector is as short as possible.

The only tubing connections still required are:

- **From the main solvent flask to the input at T-Piece 1a**
- **From the calibration fluid flask to the input of the checkvalve at pump 3**
- **From the rotary valve port 1 to your waste bottle**
- **From the rotary valve port 3 to the MS input.**
- **From the HPLC (or BPSU - system dependant) to the splitter**
- **From the splitter to the NMR probehead**

see Figure 3.1., BNMI-HP Tubing Connections Layout on page 6 for tubing types.

At delivery all unconnected ports will have been blocked with a plug in order to protect them from contamination.

The tubing (available in the accessory kit) should be cut to length using a suitable tubing cutter (not supplied). You must ensure that the tubing ends are cleanly cut at precisely 90° and correctly attached using the fittings provided (***see Figure 6.2., Correct seating of Fittings on page 49***).The internal connections in the BNMI-HP will already have been made in-house prior to test and shipping.

Remove the plug to the rotary valve port 1 and attach the 0.5mm ID FEP tubing in its place. Cut to length and insert the other end in your waste flask.

The unit is used to pump standard solvents used in LC-MS. These can be poisonous and flammable. Only personnel schooled in the use of and aware of the dangers associated with such solvents should be handling them. Safe collection, storage and disposal of the waste is the responsibility of the user and should be done according to 'Good Laboratory Practice'.

At this stage the tubing connections to the MS input and to the BNMI-HP splitter input should not be made. Ensure that the plugs at the splitter (at main flow in and high flow out) are tight. Loosen, but ***do not*** remove the plug at rotary valve port 3.

Fill one of the flasks with your solvent. Attach a length (approx. 50cm) of FEP Tubing (0.5mm ID) to an inline filter (***see Figure 6.3., Solvent Filter on page 50***). And feed the other end of the tubing through from the inside of the cap adapter. Firmly screw the cap onto the flask and pull the tubing through such that the end of the filter sits on the base of the flask. Mount the stainless steel flask holder on top of the BNMI-HP unit and place the flask in the holder. Cut the tubing to length and put a 10-32 short fitting on to the end of the tubing. Use a syringe to degass this length of tubing (***see Figure 6.2., Degassing Tubing with a Syringe on page 49***) Attach the fitting to the T-Piece 1a.

Remove the tubing connection from the T-Piece 1b (connected to Pump2 Input valve) and replace with a plug.

Remove the outlet valve from Pump1 (Fingertight but very secure).

Attach the adapter with a 1/4 28 fitting to the syringe (***see Figure 6.2., Degassing Tubing with a Syringe on page 49*** again). Depress the syringe plunger completely and attach the 1/4 28 fitting to the Pump1 outlet.

Lift the front of the BNMI unit up a few cm. and place some item (e.g. the blue cap from the solvent flask) under the unit to hold it in position. This is to ensure that any gas in the pump chambers rises to the pump outlets.

With the syringe pointing down, slowly pull out the plunger until only liquid is being sucked into the syringe.

Disconnect the adapter from the pumphead and replace the outlet valve.

Repeat this procedure for Pump 2.

Repeat also for Pump 3. However, it gets its own solvent (calibration fluid) flask and filter.

Once you are finished ensure that the tubing from the flasks to the pumps is connected as shown. (***see Figure 3.1., BNMI-HP Tubing Connections Layout on page 6***)

Now Connect the ethernet cable between the ethernet switch and the ethernet connector at the rear of the BNMI-HP

The cable can be routed around the rear of the MS unit and into the rear of the LC-NMR cabinet and then to the ethernet switch. With the unit switched off (mains switch ’0’ depressed) connect a mains lead to the rear of the unit. See ***page 7, Figure 3.2.BMNI-HP Rear View showing Electrical Connections*** The Debug RS232 connection is needed only for debug and service and is normally not connected.

Power up the unit. *3.4.1*

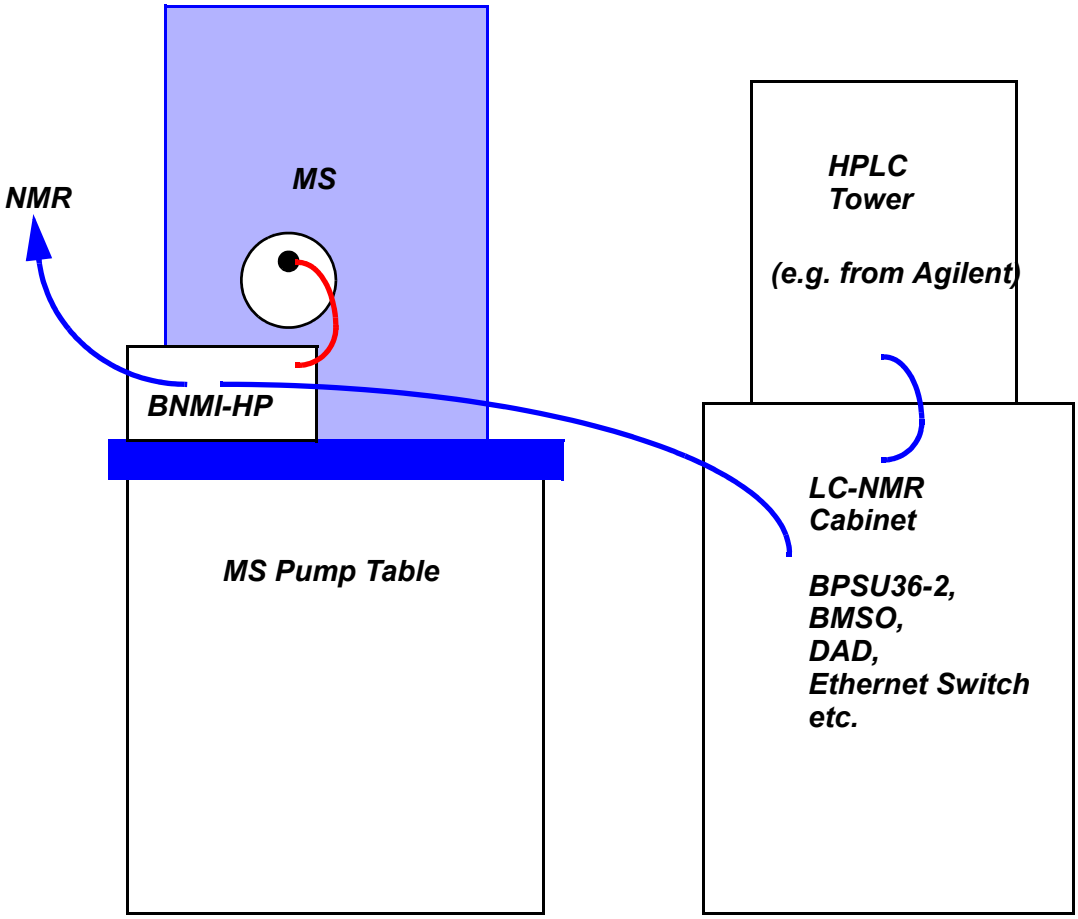
Depress the mains switch ’1’ to power up the unit.

The Status LEDs show in sequence 0000011 (MSB at top, 1 means LED lights) 00000000 to 11111100 counting through only the top 6 (green) LEDs, 11000000, then 00000001, 00000010 ..... 10000000 twice (i.e. each LED lit in turn) finishing on 10000000 indicating that the control board is running in application mode. Some 20-30 secs. later this changes to 11000000 indicating that the ethernet controller is now up and running.

Start a web browser on a PC/Laptop attached to the switch. The network settings for the associated network card **must** be set to a free (not used by anything else in the control network) IP address in the range 192.168.254.xxx. If the PC used is the HyStar controller then xxx is normally 1. The subnetmask should be set to 255.255.255.0

Start the browser of your choice and enter the url. 192.168.254.42. in the address line. This is the home page of the BNMI-HP and is also the control page for the main functions of the unit (see ***"Main Functions Page: service.html" on page 15***)..

Figure 3.3. LC-NMR-MS System: Tubing Interconnections



The BNMI-HP was tested in-house using Isopropanol (HPLC Grade). Before running any experiments the pumps have to be primed and all traces of the test fluid washed out.

On the PC-Browser, displaying the 'Main Functions' page left-click on the 'Initialise Hardware' button. This initialises the rotary valve and the 3 pumps in turn and finishes with the rotary valve in the 'waste' position. Click on 'main Functions / Refresh Page' at any time to update the information displayed. Do not use the browser's 'back' button.

Replace the plug on Port 3 of the rotary valve with a 10-32 fitting and length of FEP tubing which is fed to your waste bottle. This is a temporary connection only needed while all the pumps are washing.

With the rotary valve at the 'waste' (= init = calib) position the flow from the double syringe pump (pumps 1 and 2) flows into waste and the flow from the calibration pump flows to the output to the MS - which is at present also connected to waste.

On the PC-Browser, displaying the 'Main Functions' page left-click on the 'Double Syringe Pump' link. This opens the 'Gradient Entry' page. In the column labelled 'New Gradient' enter 250 in the 'Final Flowrate' row and click on the 'Enter New' button. This sets the double syringe pump flowrate to its maximum of 250µL/min.

Click on the link 'Main Functions' to return to the previous menu.

Click on the 'Start' button to start the double syringe pump.

Now click on the 'Service' link at the bottom of the page to enter the main service menu.

Click on the 'pump3 test' link to enter the pump 3 test page.
(see ***"Low Level Operation: pump3.html" on page 24***)

In the 'Pump Flowrate' row enter 250 and click on the button 'Enter new Flowrate'.

In the 'Suck Flowrate' row enter 500 and click on the button 'Enter new Flowrate'.

Click on the 'Start Pump3' button to start the calibration pump.

Ensure that fluid is being pumped: it should slowly drop from the tubing at the waste flask.

Leave the pumps running for ca. 1 - 2hours to thoroughly flush the system.

When this is completed return to the 'Main Functions' menu and click on the 'Emergency Stop' Button to halt the process.

Remove the tubing from Port 3 of the rotary valve to waste and replace it with the correct tubing type and connect it to the MS input.

You can now start HyStar on your controller PC. See the HyStar manual for further details.

Embedded Web Server

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HTML Pages 4.1

For service and test the BNMI-HP can be manually controlled using its HTML web pages. These pages all link through to one another or can be accessed directly by entering the address into the browser address line as follows

IP-address/page name

e.g

192.168.254.42/ews.html

Root Page: ews.html 4.1.1

This is accessed by entering the address or by clicking on the link main here or in any of the other html pages.

Figure 4.1. ews.html

This page simply provides links to further web pages.

Service Page: service.html *4.1.2*

This is accessed by entering the address or by clicking on the link service here, or in any of the other html pages.

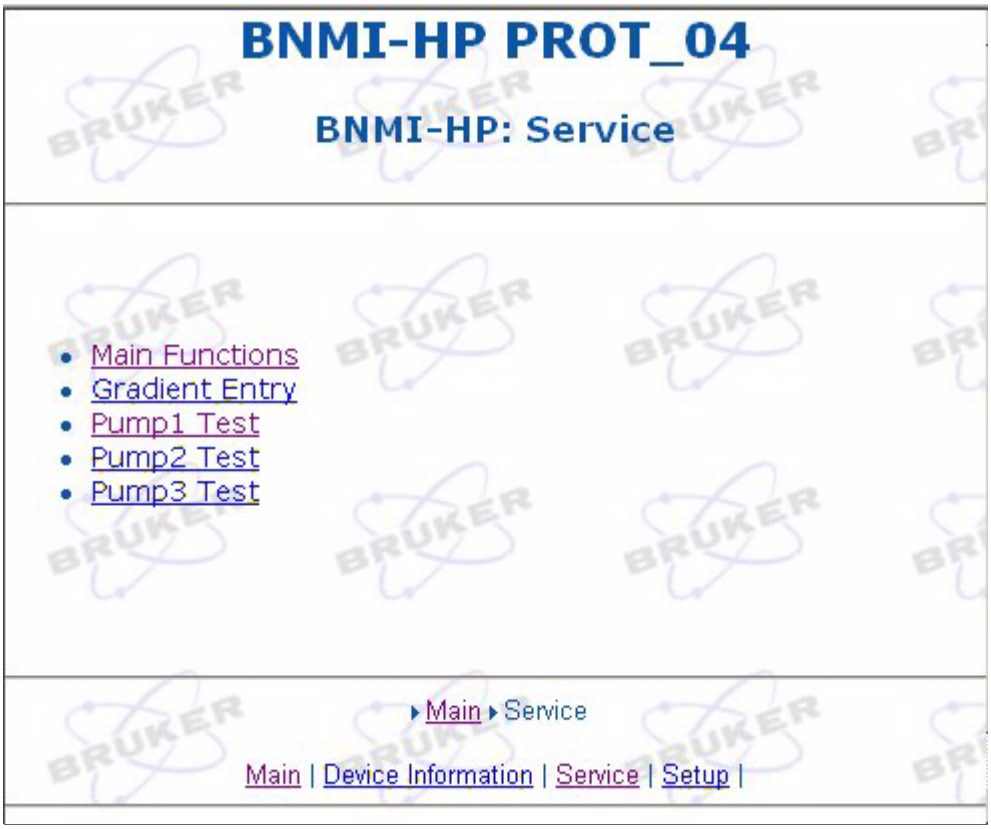


Figure 4.2. service.html

This page provides links to those web pages from which you can operate and test the BNMI-HP.

Main Functions Page: service.html *4.1.3*

This is accessed by entering the address or by clicking on the link Main Functions here, or in any of the other html pages. (This is also the home page)

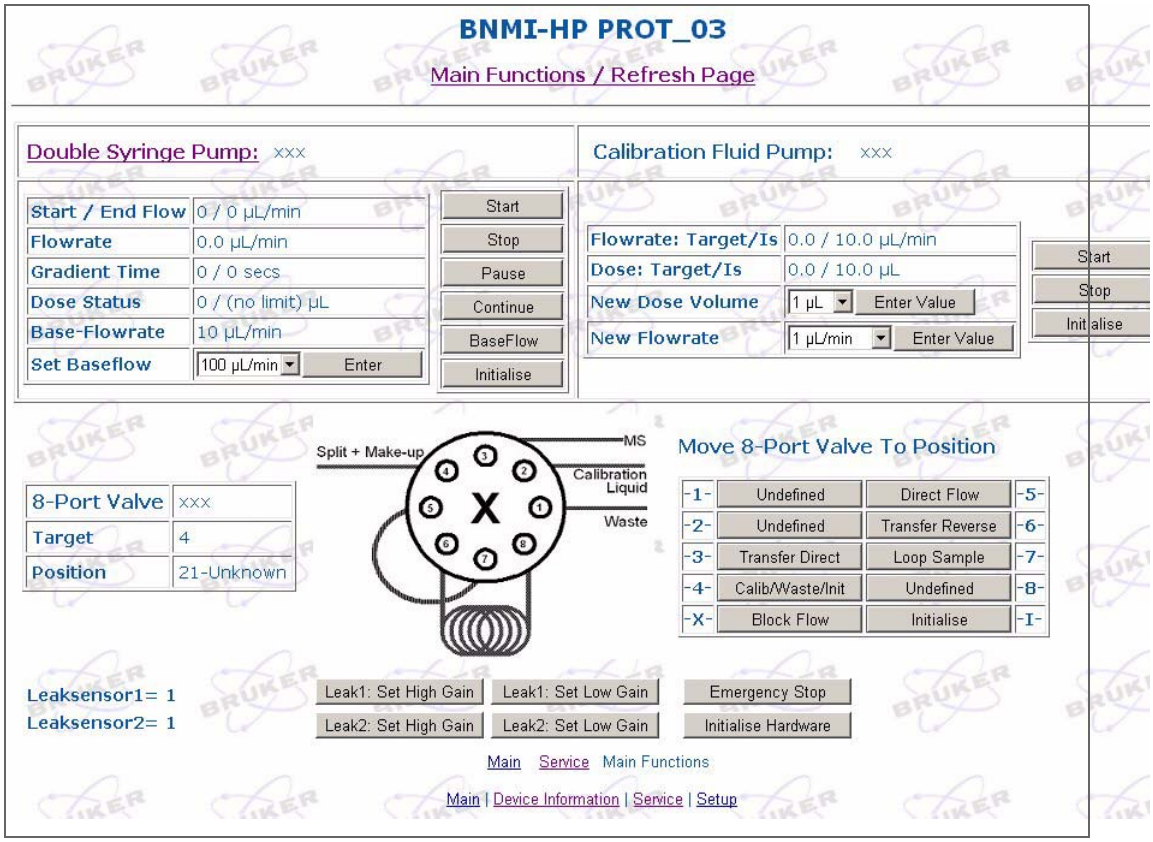


Figure 4.3. bnmi.html

From this page you can control and monitor many of the standard BNMI-HP operations.

Main Functions: Reloads the present page.

Double Syringe Pump: Clicking on the label opens a page in which you can enter new gradient data for the double syringe pump.

The text to the right of the label shows the present status of the double syringe pump.

xxx	unknown (e.g. after a reset)
end	stopped (home seek was previously successfully found in both pumps)
init	one or both pumps is in the process of initializing (home seek)
run	the pump operation is in progress.
err	stopped because an error has occurred
rdy	the pump is running in 'baseflow' mode
pause	the pump was running and has been paused
test	one of or both the pumps is in single pump (test) mode

The table below Double Syringe Pump: shows further information regarding the operation of the pump.

Start / End Flow:	Shows the start and final flowrates for the present gradient.
Flowrate:	Shows the flowrate at present.
Gradient Time:	Shows the amount of time gone and total time for the present gradient.
Dose Status:	Shows the volume dosed so far and the target volume since last start.

The buttons to the right of the table allow you to initiate the following operations:

Start	Starts the double syringe pump using the first gradient in the list. The cumulative dose volume is reset to 0. (Dose Status above). If no gradient data is available then the pump does not start. NOTE: If you start the double syringe pump before both pumps have successfully completed a home seek (initialisation) then the initialisation operation will be started first.
Stop	Stops the double syringe pump operation. The present gradient is deemed to be completed and the next gradient becomes the first gradient in the list.
Pause	Pauses the double syringe pump operation, saving the present gradient information.
Continue	Starts the pump from where it had been paused. If the pump had been stopped then simply starts using the first gradient in the list. The cumulative dose volume counts from its present value and is not reset. NOTE: If you continue (start) the double syringe pump before both pumps have successfully completed a home seek (initialisation) then the initialisation operation will be started first.
Baseflow	The pump immediately switches to Baseflow mode. This is a low flowrate (default is 10µL/min. but is programmable) which would normally be pumping continuously before an experiment is started or for example during the 'stopped' phase of a stop-flow run. If the pump had been running then the present gradient values are saved as in 'Pause' above.
Initialize	Stops the double syringe pump and initiates a home seek operation in each of the pumps. Beware: The 8-Port valve is NOT automatically set to a suitable position! (e.g. waste)

Calibration Fluid Pump:	The text to the right of the label shows the present status of the double syringe pump.
xxx	unknown (e.g. after a reset)
end	stopped (home seek was previously successfully found in the pump)
init	the pump is in the process of initializing (home seek)
run	the pump operation is in progress.
err	stopped because an error has occurred
test	the calibration pump is in single pump (test) mode

The table below Calibration Fluid Pump shows further information regarding the operation of the pump.

Flowrate: Target/Is	Shows the demand and actual flowrates for the pump.
Dose: Target/Is	Shows the demand and actual dose volume.
New Dose Volume:	Here you can select a new demand dose volume from the list
Dose Flowrate:	Here you can select a new flowrate from the list

The buttons to the right of the table allow you to initiate the following operations:

Start	Starts the calibration pump using the Flowrate and dose as defined above. NOTE: If you start the calibration pump before it has successfully completed a home seek (initialisation) then the initialisation operation will be started first.
Stop	Stops the calibration pump operation. This is not normally necessary as the calibration operation stops automatically once the target volume has been dosed.
Initialize	Stops the calibration pump and initiates a home seek operation. Beware: The 8-Port valve is NOT automatically set to a suitable position! (e.g. Transfer Direct or Reverse: Calibration fluid flows to waste)

8-Port Valve:	The text to the right of the label shows the present status of the 8-Port Valve
xxx	unknown (e.g. after a reset)
end	stopped (home seek was previously successfully found in the pump)
init	the valve is in the process of initializing (home seek)
run	the valve move operation is in progress.
err	stopped because an error has occurred

Operation of the 8-Port Valve

The table below 8-Port Valve shows further information regarding the operation of the valve.

Target	Shows the target position for the valve (simply 1 to 8).
Position	Shows the actual position of the valve (1 to 8 as well as the description).

The diagram to the right of the table displays the present position of the valve with the intended flow path and direction indicated by the blue arrows. (red for the calibration fluid)

The buttons to the right of the diagram allow you to initiate the following operations:

1	Undefined	Moves the valve to position 1: Not defined for use in any BNMI-HP operation
2	Undefined	Moves the valve to position 2: Not defined for use in any BNMI-HP operation
3	Transfer Direct	Moves the valve to position 3: Used by the BNMI-HP in Transfer Direct mode
4	Calib/Waste/Init	Moves the valve to position 4: Used by the BNMI-HP in Calibration, Waste and Initialisation (startup default) modes.
5	Direct Flow	Moves the valve to position 5: Used by the BNMI-HP in Direct Flow mode.
6	Transfer Reverse	Moves the valve to position 6: Used by the BNMI-HP in Reverse Transfer mode.

7	Loop Sample	Moves the valve to position 7: Used by the BNMI-HP in Loop Sample mode.
8	Undefined	Moves the valve to position 8: Not defined for use in any BNMI-HP operation
X	Block Flow	Moves the valve offset from the present position such that all ports are blocked: This is shown numerically as Posn+10. (i.e. 3 become 13 etc.)
X	Initialize	Initiates the valve home seek operation. Sets the valve to position 4 (Init) when finished.

Leaksensor1 = x	The value 'x' to the right of the label is a number between 0 and 99 indicating the level of conductivity of the leaksensor. A leak is flagged when this analog value exceeds 30 for more than 20 seconds continuously. As son as the value falls below 30 then the leak is no longer flagged. (Bottom Red LED and in status.xml page)
Leaksensor2 = x	Operates identically to Leaksensor1 but is, in any case, not connected at present.

Leak1:Set High Gain	Sets the amplifier gain for leak sensor 1 to Low (default)
Leak1:Set Low Gain	Sets the amplifier gain for leak sensor 1 to High (ca. x5 more than Low)
Leak2:Set High Gain	Sets the amplifier gain for leak sensor 2 to Low (default)
Leak12Set Low Gain	Sets the amplifier gain for leak sensor 2 to High (ca. x5 more than Low)
Emergency Stop	Kills all Pump and valve control Tasks immediately, disables pump and valve driver circuitry. Almost a reset but not quite.
Initialise Hardware	ICarries out the following sequence of operations:-

- **initialises the rotary valve (if required)**
- **sets rotary valve such that calibration pump is directed to waste.**
- **initialises the calibration pump (if required)**
- **sets the rotary valve such that the double syringe pump is directed to waste.**
- **initialises the 2 pumps for the double syringe pump (if required).**
- **sets valve to 'direct' position ready for normal operation.**

Gradient Entry Page: operate.html *4.1.4*

This is accessed by entering the address, by clicking on the link Gradient Entry here, or in the service.html page or via Double Syringe Pump:. in bnmihp.html.

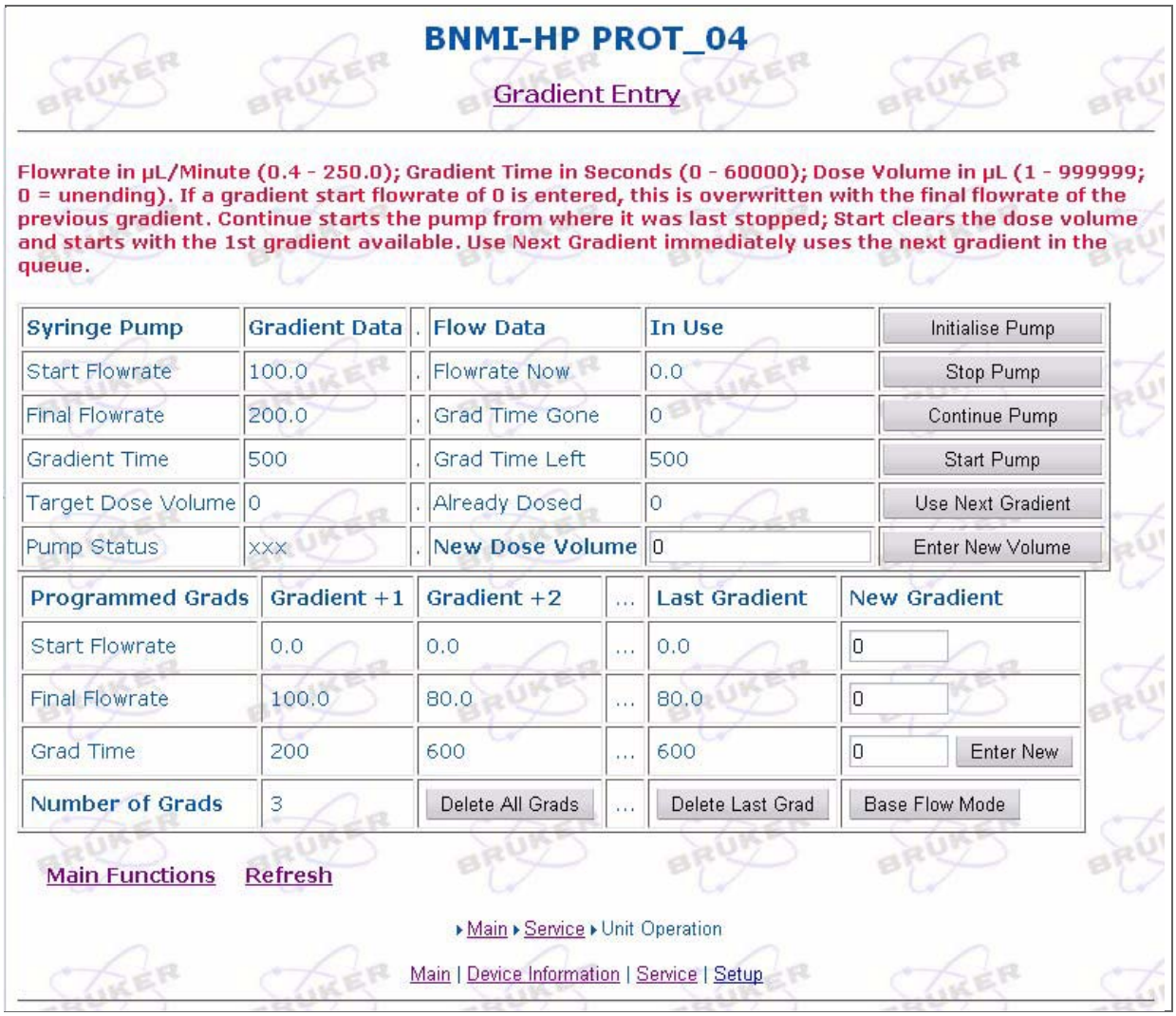


Figure 4.4. operate.html

This page is primarily intended for entering new gradient data

Initialize Pump — Stops the double syringe pump and initiates a home seek operation in each of the pumps. Beware: The 8-Port valve is NOT automatically set to a suitable position! (e.g. waste)

Stop Pump — Stops the double syringe pump operation. The present gradient is deemed to be completed and the next gradient becomes the first gradient in the list

Continue Pump — .Starts the pump from where it had been paused. If the pump had been stopped then simply starts using the first gradient in the list. The cumulative dose volume counts from its present value and is not reset. NOTE: If you continue (start) the double syringe pump before both pumps have successfully completed a home seek (initialisation) then the initialisation operation will be started first.

Start Pump	Starts the double syringe pump using the first gradient in the list. The cumulative dose volume is reset to 0. (Dose Status above). If no gradient data is available then the pump does not start. NOTE: If you start the double syringe pump before both pumps have successfully completed a home seek (initialisation) then the initialisation operation will be started first.
Use Next Gradient	Finishes the present gradient flow operation immediately and jumps to the next gradient.
Enter New Volume	Loads a new dose volume limit (1 - 999999µL, 0 = endless)
Enter New	Enter the start flowrate (µL/min.) the final flowrate and the gradient time (seconds) into the fields to the left if this button and click on the button to load the values
Delete all Grads	Deletes all gradient information from memory.
Delete last Grad	Deletes the most recent gradient information from memory.
Base Flow Mode	The pump immediately switches to Baseflow mode. This is a low flowrate (default is 10µL/min. but is programmable) which would normally be pumping continuously before an experiment is started or for example during the 'stopped' phase of a stop-flow run. If the pump had been running then the present gradient values are saved as in 'Pause' above.
Main Functions	Loads the Main Functions page.
Refresh	Reloads the present page.

Low Level Operation: pump1.html *4.1.5*

This is accessed by entering the address, by clicking on the link pump1 test here, in the service.html page or via Pump1 in pump2.html and pump3.htm

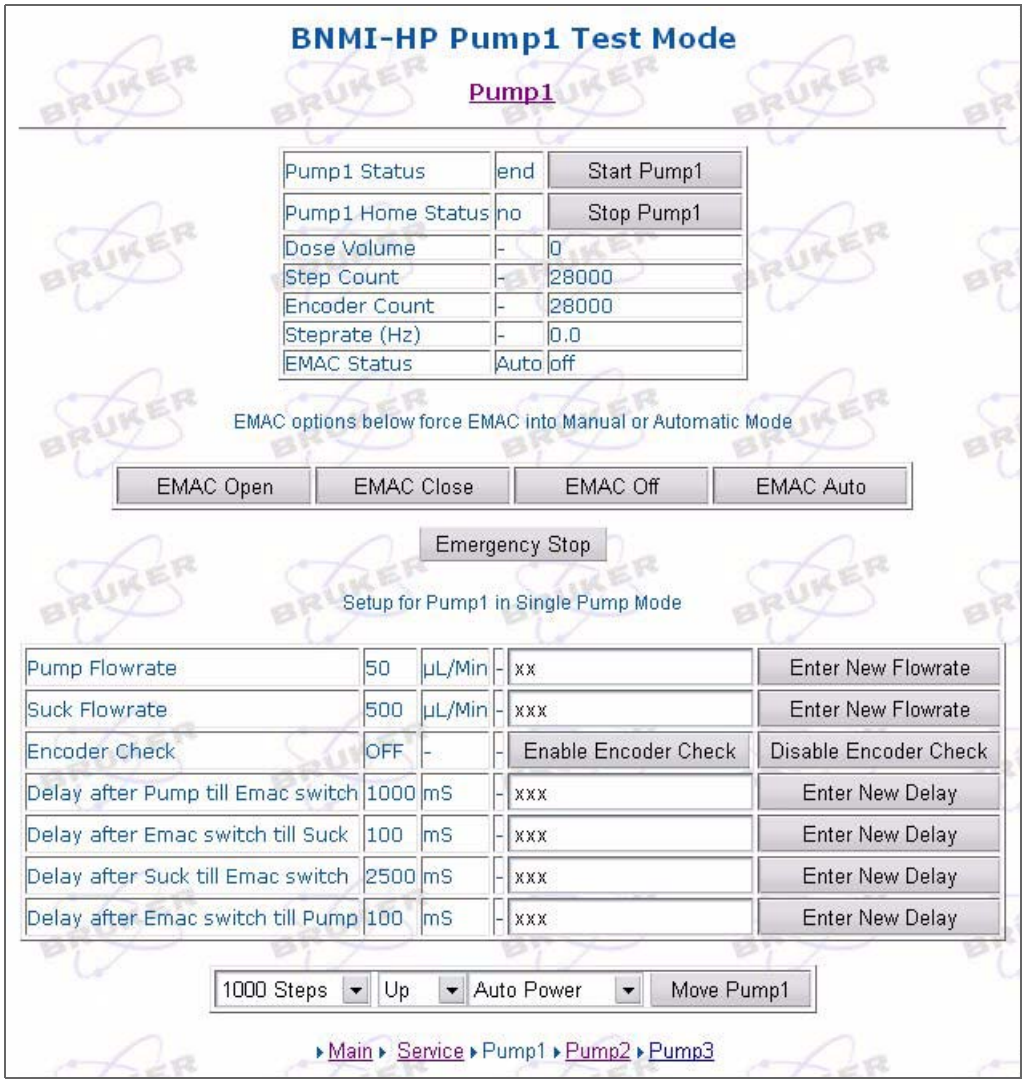


Figure 4.5. pump1.html

This is page intended as an aid to test service and installation.

Pump1 Reloads this page

Upper Table Entries:

Pump 1 Status	
xxx	unknown (e.g. after a reset)
end	the pump has stopped
suck	the pump is sucking fluid in
pump	the pump is pumping out
err	stopped because an error has occurred
Pump1 Home Status	
no	The home sensor has not yet been found
ok	The home sensor was already found
Dose Volume	shows the volume (µL) pumped out since the 'Start Pump1' button was pressed.
Step Count	shows the value of the internal step counter.
Encoder Count	shows the value of the internal encoder counter.
Steprate (Hz)	shows the steprate in use at present for pump1 stepmotor. (The nominal Flowrate in µL/min. = steprate/4)
Emac Status	NOTE: The EMAC valves are electromagnetically actuated non-return valves. This means that in the 'open' position the non-return function is effectively disabled and the fluid can flow in both directions. This is used to ensure that the fluid flows easily into the valve during a 'suck' phase of the pump. In the closed position the valve is forced into non-return mode. This ensures that no fluid flows back into the solvent flask during the 'pump out' phase. However fluid would still be able to flow into the valve. (e.g. during purging, while the pump is pumping you can suck additional fluid through the pump using a syringe). In the 'Off' mode if the pump is pumping out and the EMAC had been actively closed then the back pressure from the pump should be sufficient to keep the valve closed (non-return mode). However correct operation could not be guaranteed.
Auto	means the Electromagnetically actuated inlet valve is opened and closed automatically during the pump routine.
manual	the valve is opened or closed manually using the buttons below this table
Start Pump1	The EMAC is closed and the pump starts to pump out using the setups and delays as defined in the table below. If the EMAC is in 'Auto' mode then it is opened/ closed as required. If the home sensor has not yet been found then the step and encoder counters are first set to there minimum values (= fully sucked in position). The pump pumps out until the home sensor is found where the counters are set to 'HOME' position values and the Home Status is set to ok. the pump continues to suck/pump endlessly until stopped. As long as the home sensor has **NOT** yet been found then the 'pump out' flowrate is limited to 50µL/min.
Stop Pump1	The pump is stopped immediately. (Only if had been started in test mode here)

Operation of the EMAC buttons

EMAC Open	Opens the Electromagnetically actuated inlet valve.

EMAC Close	Closes the Electromagnetically actuated inlet valve (forces non-return mode)
EMAC Off	Removes power to the Electromagnetically actuated inlet valve.
EMAC Auto	The valve is operated automatically during the pumping routing.
Emergency Stop	Kills all Pump and valve control Tasks immediately, disables pump and valve driver circuitry. Almost a reset but not quite.

Function of the bottom table

The values in column 2 of the table have the following meaning:

Pump Flowrate	Shows the present ***Target*** 'pump out' flowrate.
Suck Flowrate	Shows the present ***Target*** 'suck in' flowrate.
Encoder Check	OFF or ON shows if the encoder monitor function is enabled.
Delay after Pump till Emac switch	At the end of the 'pump out' phase the pump waits xxxxmS before switching the Emac from closed to open.
Delay after Emac switch till suck	After the 'pump out' phase and after switching the Emac from closed to open the pump waits a further xxxxmS before starting the 'suck in' phase.
Delay after Suck till Emac switch	At the end of the 'suck in' phase the pump waits xxxxmS before switching the Emac from open to closed.
Delay after Emac switch till pump	After the 'suck in' phase and after switching the Emac from open to closed the pump waits a further xxxxmS before starting the 'pump out' phase.

The buttons operate as follows: (from top to bottom)

Enter new Flowrate	The value written in the column to the left of the button is set to be then new 'pump out ' flowrate. (only valid once Home Status = ok) range 1..... 250
Enter new Flowrate	The value written in the column to the left of the button is set to be then new 'suck in' flowrate. range 2.....500 (increments of 2)
Enable Encoder Check	The encoder check is enabled. The stepmotor counter and the encoder counter are continuously checked against each other. If a discrepancy of more than a few hundred counts is found then the pump is stopped, the counters reset (to maximum or minimum depending on the direction of the last move) and an error is flagged. On Power up and after reset this mode is disabled. After the pump has successfully completed the 'home seek' initialisation routine the encoder counter is checked against the step counter. If these are the same (within tolerance) then the encoder check is automatically enabled.
Disable Encoder Check	The encoder check is disabled.
Enter new Delay	The 'Delay after Pump till Emac switch' is set to the value written in the column to the left of the button. (0 - 2500mS, resolution 10mS)
Enter new Delay	The 'Delay after Emac switch till suck' is set to the value written in the column to the left of the button. (0 - 2500mS, resolution 10mS)

Enter new Delay

The ’Delay after Suck till Emac switch’ is set to the value written in the column to the left of the button. (0 - 2500mS, resolution 10mS)

Enter new Delay

The ’Delay after Emac switch till pump’ is set to the value written in the column to the left of the button. (0 - 2500mS, resolution 10mS)

The Move Pump Option

Move Pump1

The Pump is moved the number of steps (1000, 5000, 10000 or 250000) in the direction (Up/Down = Pump/Suck) and with the power mode selected in the options to the left of the button. The step speed is taken from the flowrates defined and used for pump/suck defined above.

Low Level Operation: pump2.html *4.1.6*

This functions for exactly as in ***"Low Level Operation: pump1.html" on page 21*** but controls Pump2 .

Low Level Operation: pump3.html *4.1.7*

This functions exactly as in ***"Low Level Operation: pump1.html" on page 21*** but controls Pump3 .

Ethernet Interface for Automated Control 4.2

'Forms' based html webpages as used for service and test are unsuitable for automated control. The control program, HyStar, communicates with the unit by sending commands and data embedded in the URL address and reads status information from the XML web pages generated by the embedded web server.

Embedded URL Commands 4.2.1

All commands are in the form $XXX=aaa, where '$' flags that a command follows, **XXX** is the command (the command length is not fixed) and aaa is the data or operation belonging to the command.

The following commands are normally only issued by HyStar. However they will work in manual mode. Simply access the status.xml webpage (Not all browsers correctly display xml pages. Internet Explorer does work here). In the URL Address line replace the text 'status.xml' with the command you wish to have carried out. (e.g. http://192.168.254.42/$PUMP=start)

The unit responds with one of the following 2 webpages

Figure 4.6. Command Response: valid/invalid command syntax

AOK means the command syntax was OK. It does not mean the command has been implemented. Any problems due to the command will show up later in the status.xml webpage. (see ***"XML Webpages" on page 30***)

ERR means the command was not entered correctly and will not be carried out

Double Syringe Pump Operation

Command	Operation	Comment
$PUMP=start	Starts double syringe pump (uses the first entry in the gradient table)	Status Line: <RUN>run</RUN> The volume already dosed is reset to 0. If no gradient data is available then the pumps do not start.
$PUMP=init	Pump1 and Pump2 , which make up the double syringe pump carry out their initialisation routine (find home sensor then suck in)	Status Line: <RUN>init</RUN> You should only run this at start-up or test.
$PUMP=pause	The present pump status is saved and the pump stops.	Status Line: <RUN>pause</RUN>
$PUMP=continue	The previously saved values from 'pause' are restored and the pump runs again.	Status Line: <RUN>run</RUN>
$PUMP=halt	The pump operation is stopped. The present gradient is aborted and the associated data deleted.	Status Line: <RUN>end</RUN>
$PUMP=next	The first or present gradient is replaced by the next gradient in the list.	Status Line: unchanged.
$PUMP=on	The pump starts immediately at the Base Flowrate. (independent of any gradient data) See $BASEFLOW below. The 'Dose' counter does not count. If the pump was already running then as in $PUMP=pause the present gradient data is saved.	Status Line: <RUN>rdy</RUN>

NOTE: In the event that only one gradient had been defined or the pump was already using the last gradient data set: If the Pump is stopped with $PUMP=halt this gradient data set is deleted and the pump can only pump again after fresh gradient data have been loaded. But if the pump is stopped with $PUMP=pause their are 2 options to restart the pump:

1) $PUMP=continue: The pump continues from where it was when it was stopped.

2) $PUMP=start: The pump starts at the beginning of the first available gradient data set. (i.e. the 'paused' gradient will be restarted.)

Gradients and Dosing

Command	Operation	Comment
$STARTFLOW=xxx	xxx = 0 to 250. Values less than 0.4 are replaced with 0.0. Resolution = 0.1. e.g. 123.4 is 123.4µL/Minute	Default Value is 'ENDFLOW' from the previous gradient.
$GRADTIME=xxx	xxx = 0 to 60000. values above 60000 are replaced with 60000. Resolution is 1 Sec.	Default Value = 0. This results in a step change in flowrate when the new gradient is used
$ENDFLOW=xxx	xxx = 0 to 250. Values less than 0.4 are replaced with 0.0. Resolution = 0.1. e.g. 123.4 is 123.4µL/Minute	(Only) On receiving this command the gradient values are written into the table.
$BASEFLOW=xxx	xxx = 0 to 250. Values less than 0.4 are replaced with 0.0. Resolution = 0.1. e.g. 123.4 is 123.4µL/Minute	Default = 10µL /Sec. This is the static pre-run flowrate for the pump.
$DOSEVOL=xxx	xxx= 0 to 999999. Default value 0 means unending. Resolution = 1 (µL)	The pump operation stops after it has dosed ≥ xxxµL.
$DELGRAD=last	The last (most recent) data set written to the gradient table is deleted.	If the pump is already running using this data it will continue to run.
$DELGRAD=all	Deletes all data sets in the gradient table.	If the pump is already running it will continue to run.

Calibration Pump Operation

Command	Operation	Comment
$CALIBPUMP=start	Start the calibration pump operation.	Status Line: <RUN>run</RUN> The dose volume is cleared to 0.
$CALIBPUMP=init	The calibration pump carries out its initialisation routine (Home sensor seek followed by suck in)	Status Line: <RUN>init</RUN>
$CALIBPUMP=halt	Stops the pump.	Status Line: <RUN>end</RUN>
$CALIBFLOW=xxx	xxx = 0 to 250. Values less than 0.4 are replaced with 0.0. Resolution = 0.1. e.g. 123.4 is 123.4µL/Minute	Demand flowrate for the calibration pump.
$CALIBDOSE=xx	xxx= 0 to 6500.0. Default value = 200. Resolution = 0.1 (= 0.1µL). xxx = 123 means a dose volume of 12.3µL. xxx= 0 means dose endlessly.	Once it has dosed ≥ xxxµL, the pump stops and sucks fluid in again ready for the next calibration operation.

Operation of the 8-Port Valve

Command	Operation	Comment
$VALVEPOSN=xx	xx = 1 to 8 or 11 to 18. This defines the demand position for the valve. Posn. 11-18 are 'In-Between-Positions' near to posns. 1-8 but with the flow blocked.	The valve moves immediately to the demand posn. Status Line: <RUN>run</RUN>
$VALVEPOSN=direct	The valve moves immediately to the 'direct' Posn (= 6).	Status Line: <RUN>run</RUN>
$VALVEPOSN=init	The valve moves immediately to the 'init' Posn (= 5)	Status Line: <RUN>run</RUN>
$VALVEPOSN=waste	The valve moves immediately to the 'waste' Posn (= 5 = calib)	Status Line: <RUN>run</RUN>
$VALVEPOSN=calib	The valve moves immediately to the 'calib' Posn (= 5 = waste)	Status Line: <RUN>run</RUN>
$VALVEPOSN=transfer	The valve moves immediately to the 'transfer' Posn (= 4)	Status Line: <RUN>run</RUN>
$VALVEPOSN=reverse	The valve moves immediately to the 'reverse transfer' Posn (= 7)	Status Line: <RUN>run</RUN>
$VALVEPOSN=halt	The valve stops immediately	Status Line: <RUN>end</RUN>
$VALVE=clock	From now the valve will only move in a clockwise direction	
$VALVE=anti	From now the valve will only move in an anticlockwise direction	
$VALVE=direct	The valve takes the shortest route to the demand position.	Default
$VALVE=init	The valve carries out its initialisation routine (find home sensor) and then moves to the 'init' posn.	Status Line: <RUN>init</RUN>

NOTE: If a command to move the 8-Port valve to a new position is entered before the valve has carried out its initialisation routine, then the initialisation routine is first carried out then the valve moves to the new demand posn.

Miscellaneous

Command	Operation	Comment
$BNMI=init	The BNMI-HP pumps and the 8-Port valve are initialized as follows: 1)The 8-Port Valve is initialized and the moved to posn 6. This connects the output of the calibration pump to waste. 2) The calibration pump is initialized. 3) the 8-Port valve is moved to the 'waste' posn. 4)the double syringe pump (pump1 & pump2) is initialized.	The various initialisation operations are only done if needed. At start up, in the status.xml webpage wait until <BNMI>start</BNMI> appears. then send the command. You get <BNMI>init</BNMI> and finally when finished <BNMI>rdy</BNMI> or if an error occurs <BNMI>err</BNMI>
$LEAK1GAIN=low	sets gain = low for Leak sensor 1.	Default
$LEAK1GAIN=high	sets gain = high for Leak sensor 1.	
$LEAK1GAIN=none	Leak sensor 1 disabled.	
$LEAK2GAIN=low	sets gain = low for Leak sensor 2.	Default
$LEAK2GAIN=high	sets gain = high for Leak sensor 2.	
$LEAK2GAIN=none	Leak sensor 2 disabled.	
$KILL=all	All operations (pumps and valve) are immediately aborted. The pumps and the valve stop at once.	
$ERROR=ack	Error Acknowledge: errors appearing on the status.xml webpage are deleted from the message queue.	This does not fix the reason for the error it only clears the message queue.

The main Status Page 'status.xml'

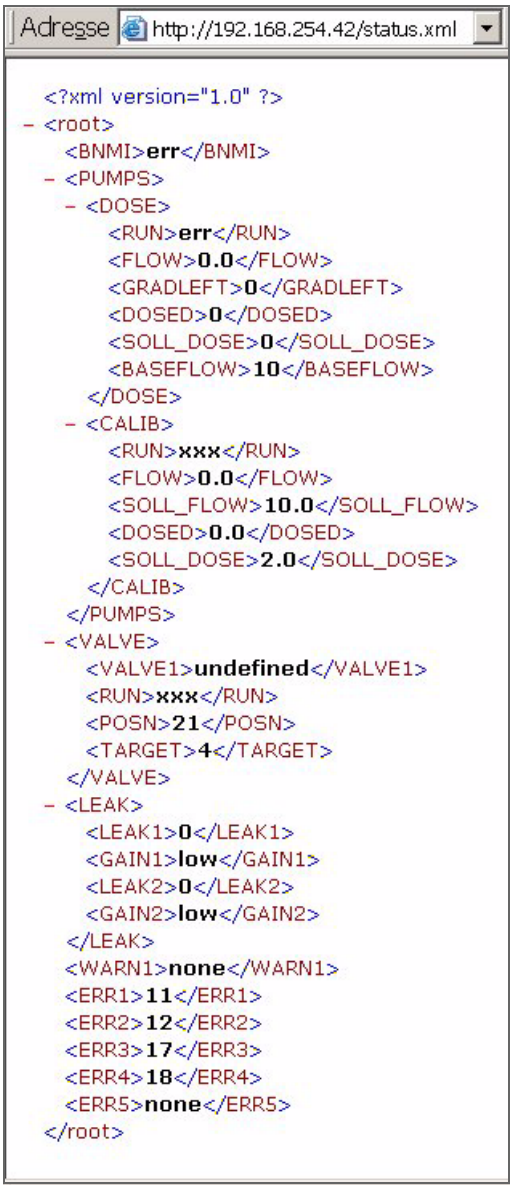


Figure 4.7. status.xml

The status page consists of a list of xml tags each containing status information for the BNMI-HP. These are parsed and logged by the Control program HyStar to allow it to monitor the status of the BNMI-HP and control the program flow.

These **Tags** have the following meaning:

<BNMI>	Overall BNMI-HP Status
xxx	Unknown/not ready, after reset or power up.
start	ready but initialisation not yet done
init	initialisation in progress
err	an error has occurred during initialisation
rdy	the BNMI-HP is ready for normal operation.
<PUMPS>	The following status refers to the pumps
<DOSE>	and then to the double syringe pump
<RUN>	Double syringe pump operation
xxx	unknown (after reset/ power up)
end	the pump operation has halted
pause	the pump operation has paused
err	an error has occurred and the pump operation has stopped
init	the initialisation operation is still in progress
run	the pump is pumping (gradient mode)
rdy	the pump is in baseflow mode
test	one or both of pump1 and pump2 is/are in test mode. Gradient pump mode is disabled.
<FLOW>	The present flowrate in µL/Minute
<GRADLEFT>	The time remaining (in seconds) until the present gradient flow reaches its final flowrate.
<DOSED>	The present flowrate in µL/Minute
<SOLLDOSE>	The demand dose volume in µL
<BASEFLOW>	The demand base flowrate in µL/min

<CALIB>	The following status refers to the calibration pump
<RUN>	Calibration pump operation status
xxx	unknown (after reset/ power up)
end	the pump has finished pumping out (it may still be sucking in fluid)
err	an error has occurred and the pump has stopped
init	the initialisation operation is still in progress
run	the pump is pumping out
test	Pump 3 is running in Test mode and the calibration pump mode is disabled.
busy	Only if was still sucking when a start pump command is entered and insufficient volume is available to dose in one syringe cycle.

<FLOW>	The present flowrate in µL/Minute
<SOLL_FLOW>	The demand flowrate in µL/Minute
<DOSED>	The volume dosed in µL since the last start pump command.
<SOLL_DOSE>	The demand dose volume in µL
<VALVE>	the following status refers to the 8-Port valve
<VALVE1>	is the present valve posn. in text form:
undefined	Position unknown (probably valve not yet initialized)
direct	The valve is in the 'direct' posn (= 5) i.e. double syringe pump connected to MS
waste	The valve is in the 'waste' posn (= 4) i.e. double syringe pump connected to waste
calib	The valve is in the 'calibration' posn (= 4) ie. calibration pump connected to MS and the double syringe pump connected to waste.
init	The valve is in the 'initialized' posn (= 4) i.e. double syringe pump connected to waste. This is the default set position after a home sensor seek. (init command)
sample	The valve is in the 'sample' posn (= 7) i.e. double syringe pump connected to the sample loop, flow out of sample loop to waste
transfer	The valve is in the 'transfer' posn (= 3) i.e. double syringe pump connected to the sample loop, flow direction same as in sample, flow out of loop to MS.
reverse	The valve is in the 'reverse transfer' posn (= 3) i.e. double syringe pump connected to the sample loop, flow direction is reversed from 'sample'; flow out of loop to MS.
<RUN>	The 8-Port valve operation status
xxx	unknown (after reset/ power up)
end	the valve has reached its demand target posn. and has stopped
err	an error has occurred and the valve has stopped
init	the initialisation operation is still in progress
run	the valve is in motion
<POSN>	The present posn. of the 8-Port valve. 1 - 8 are posns. where flow can take place (See ***"The 8-Port Valve Positions" on page 61***). Posns. 11-18 are 'In-Between' positions near 1-8 where the fluid path is blocked. Posns. 21-28 are unknown posns. where the valve has been moved before it has been initialized.
<TARGET>	Is the demand target posn for the valve. 1-8 and 11-18 as defined for <POSN> above.
<LEAK>	the following status refers to leak sensors
<LEAK1>	Leak 1 status
0	No Leak has been detected (or the sensor has been disabled)
1	A leak has ben detected (The leak condition must exist for >20secs)
<GAIN>	The leak sensors amplifiers have a variable gain selection
low	Normal low sensitivity gain
high	approx. x5 gain increase from low
off	the leak sensor check is disabled
<LEAK2>	Leak 2 status (is not connected at present, is otherwise identical to leak1)
0	No Leak has been detected (or the sensor has been disabled)
1	A leak has ben detected (The leak condition must exist for >20secs)
<GAIN>	The leak sensors amplifiers have a variable gain selection
low	Normal low sensitivity gain
high	approx. x5 gain increase from low

off	the leak sensor check is disabled
<WARN1>	Displays a warning number in the range 0 to 255. The last **Tag** always has the plain text 'none' to indicate it is the last warning.
.....	Warnings are error conditions which the BNMI-HP has been able to clear internally. However, if a particular warning is displayed often, this can be an indication of a hardware problem. The warnings are only displayed once.
.....	The next time that status.xml is displayed these warnings will be gone.
<WARNx>	See **** for the meaning of the warning number.
<ERR1>	Displays an error number in the range 0 to 255. The last **Tag** always has the plain text 'none' to indicate it is the last error.
.....	These are major error conditions which the BNMI-HP is not able to clear internally. The errors will not be cleared from the status page until the acknowledge error command is sent. Acknowledging the error does not clear or fix the source of the error. This may require user or service personnel intervention.
.....	See the command at ***"Miscellaneous" on page 29***
<ERRx>	See **** for the meaning of the error number.

The Information Webpage info.xml

Figure 4.8. info.xml

The status.xml webpage would normally only be accessed while the BNMI-HP is powering up or has been reset. After a reset the BNMI-HP controller interrogates the hardware in order to determine the hardware configuration and the firmware levels in use. This information is in turn read by the Ethernet Module for display here. The **Tags** here have the following meaning.

<START>	Shows the power up status as follows
NO	The unit is not yet ready
RDY	The unit is ready and only now should the remaining **Tags** be read.
<MODE>	Shows the control board operation mode
APPL	The control board is in application mode
BOOT	The control board is in Boot mode and will only run once new firmware is downloaded.
<HARDWARE>	The following Tags display information about the BNMI-HP hardware configuration.
<CONTROL_PN>	Has the P/N of the control Board
<CONTROL_SN>	Has the S/N of the control Board
<STEP1_PN>	Has the P/N of the stepmotor driver board used by pump1.
<STEP1_SN>	Has the S/N of the stepmotor driver board used by pump1.
<STEP2_PN>	Has the P/N of the stepmotor driver board used by pump2.
<STEP2_SN>	Has the S/N of the stepmotor driver board used by pump2.
<STEP3_PN>	Has the P/N of the stepmotor driver board used by pump3.
<STEP3_SN>	Has the S/N of the stepmotor driver board used by pump3

<STEP4_PN>	Has the P/N of the stepmotor driver board used by the 8-Port valve.
<STEP3_SN>	Has the S/N of the stepmotor driver board used by the 8-Port valve.
<UNIT_PN>	Has the P/N of the BNMI-HP unit.
<UNIT_SN>	Has the S/N of the BNMI-HP unit.
<CALPUMP>	has the following options
xxx	unknown, probably still in power up: wait till <START> = **RDY** above
no	The unit does not have a calibration pump
yes	The unit does have a calibration pump
<HARDWARE>	The following Tags display information about the BNMI-HP firmware configuration
<ETH_APP>	Has the release date of the application firmware in use in the ethernet module.
<CONTROL_BOOT>	Has the release date of the boot firmware in use in the control board.
<CONTROL_APPL>	Has the release date of the application firmware in use in the control board.

The Gradient Data Webpage gradient.xml

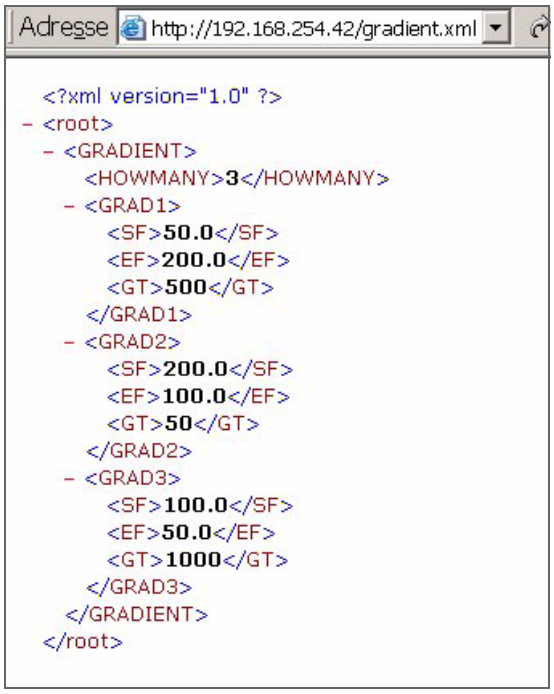


Figure 4.9. gradient.xml

The gradient.xml webpage supplies details about the gradient data saved to the BNMI-HP gradient data table.

<GRADIENT>	The information in the following **Tags** is all gradient data information.
<HOWMANY>	The number of gradient data sets in the table
<GRAD1>	Data for the first gradient dataset
to	(the number displayed in <HOWMANY>)
<GRADx>	Data for the last gradient dataset
<SF>	Is the start flowrate in µL/Min
<EF>	Is the end flowrate in µL/Min
<GT>	Is the gradient time in seconds. This is the time needed to go from the start flowrate to the end flowrate.

If the double syringe pump is running then the first data set is the gradient in use at present. If the pump is not running then when started it will use the first data set. A gradient will remain in use until its gradient time runs out. At this time it will be deleted and replaced by the 2nd data set which becomes the new first data set. All the remaining data sets also move up 1 posn.

When the gradient time for the last data set runs out then the pump continues to pump at the end flowrate until it is either stopped or a new gradient data set is loaded. The BNMI-HP can store a maximum of 255 data sets.

See ***"Gradient Pump Operation" on page 62*** for a more complete description.

Hardware 5

Mains AC Wiring 5.1

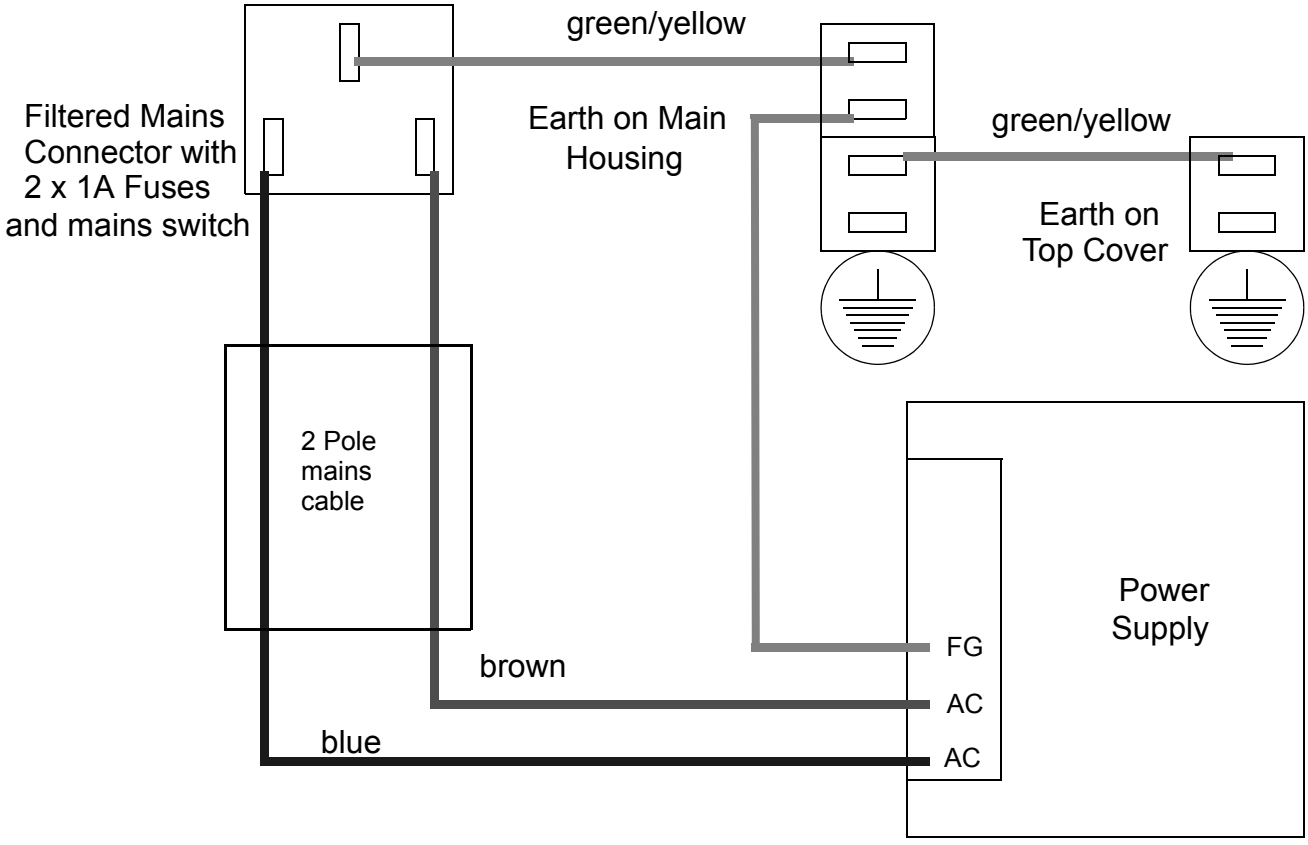


Figure 5.1. Mains AC Wiring

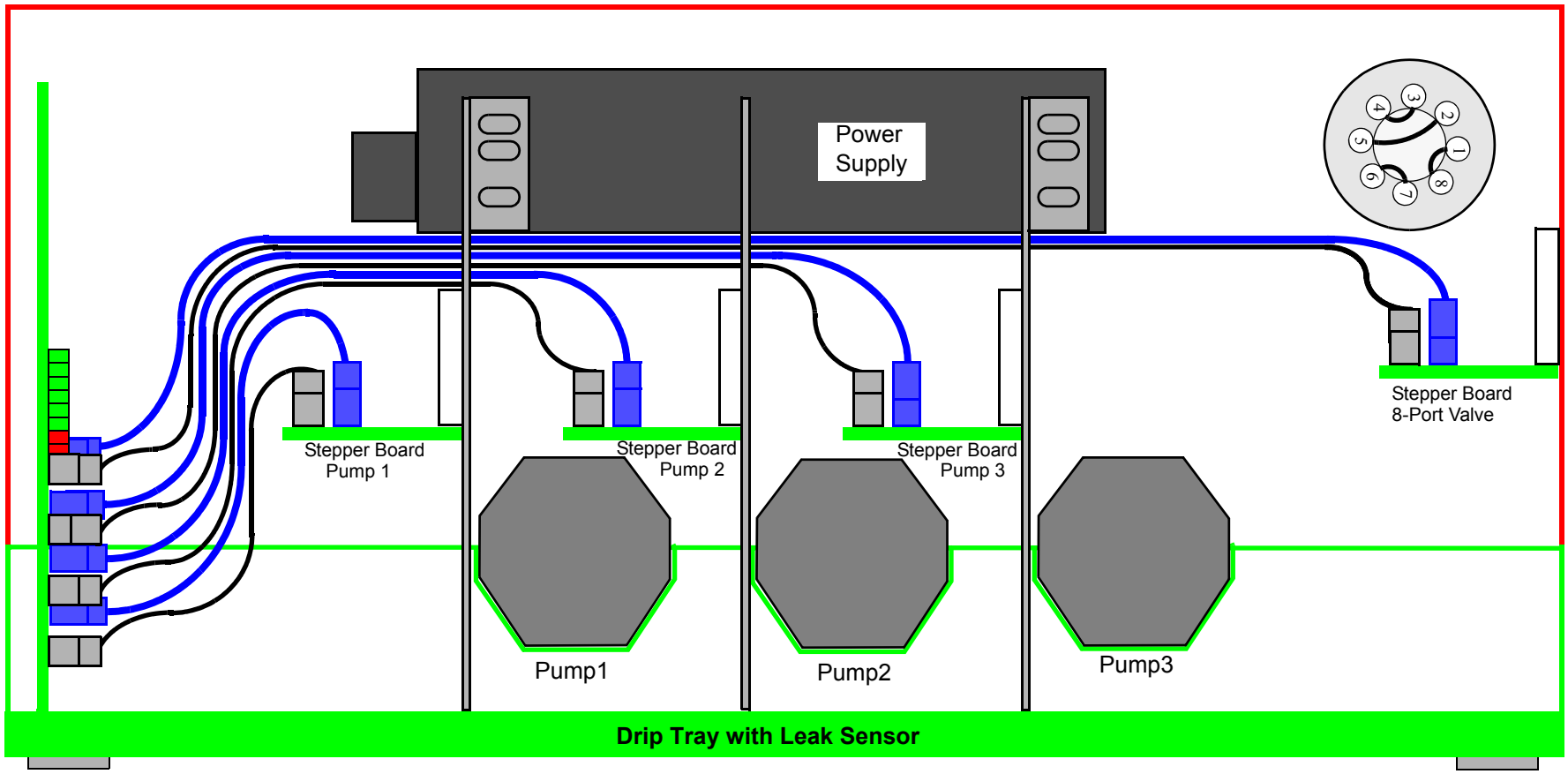


Figure 5.2. Cabling Layout, Front View

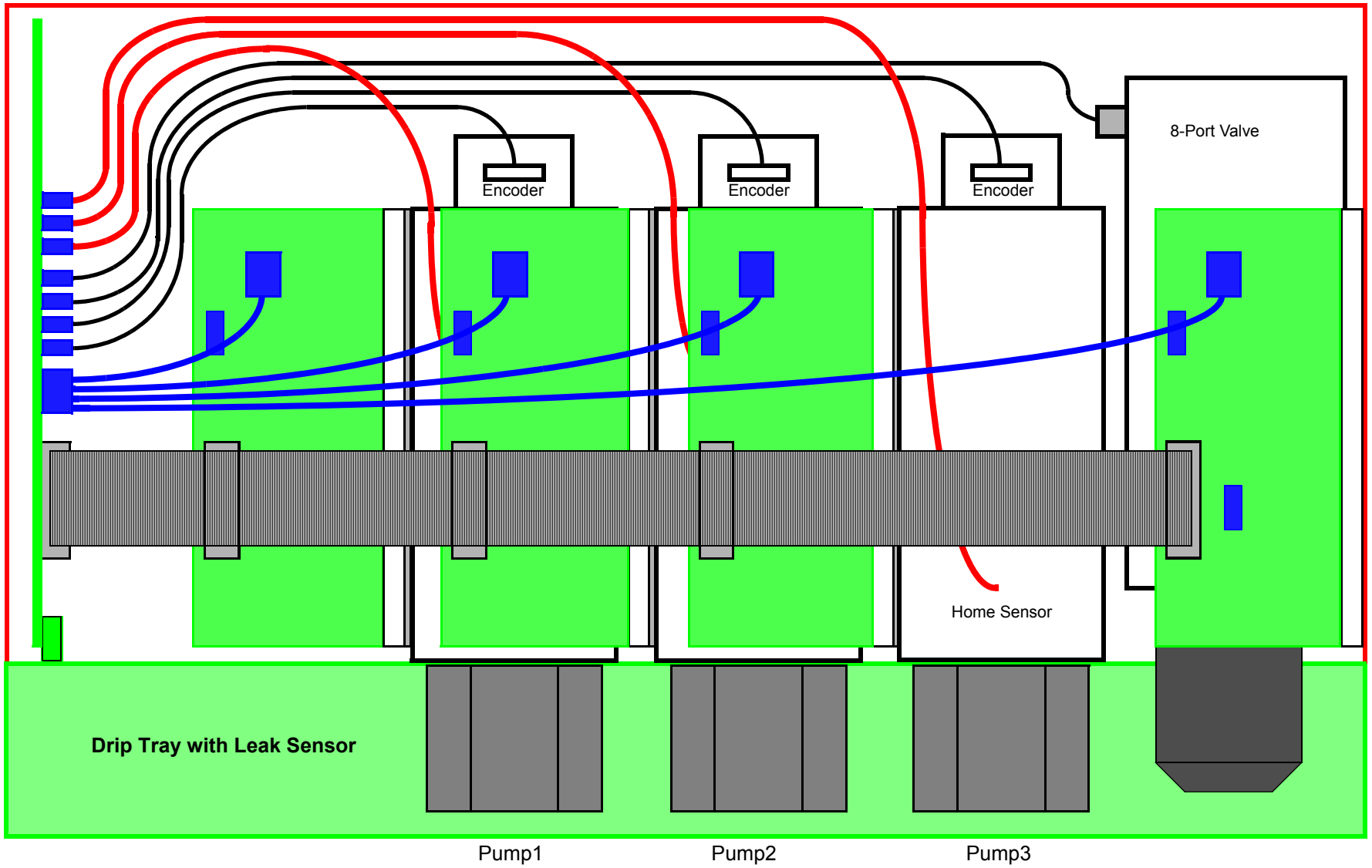


Figure 5.3. Cabling Layout, Top View

Control Board Connector and Jumper Layout 5.2

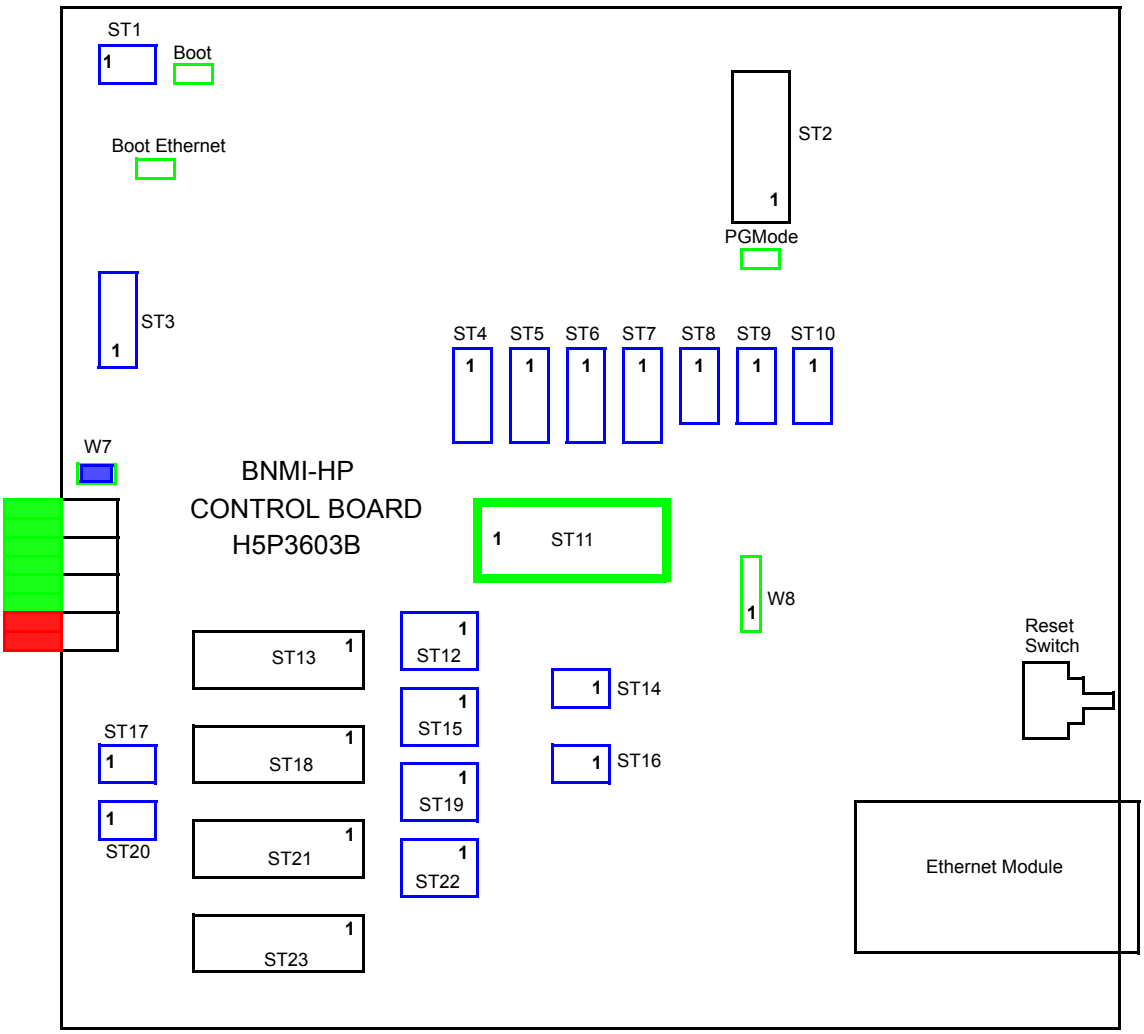


Figure 5.4. Control Board Connections

Connectors

ST1	Spare analog input intended for temperature sensor of type AD22100KT.
ST2	JTAG input for first time programming of PSD IC U3.
ST3	Spare port/analog inputs
ST4	Encoder cable from Pump 1
ST5	Encoder cable from Pump 2
ST6	Encoder cable from Pump 3
ST7	Encoder cable from 8-Port Valve (includes Home Sensor)
ST8	Home Sensor (Light Barrier) from Pump 1
ST9	Home Sensor (Light Barrier) from Pump 2

ST10	Home Sensor (Light Barrier) from Pump 3
ST11	Power Connector
ST12	Power + Rear Panel Fan Control to 8-Port Valve Stepper Driver Board
ST15	Power + EMAC Control to Pump 3 Stepper Driver Board
ST19	Power + EMAC Control to Pump 2 Stepper Driver Board
ST22	Power + EMAC Control to Pump 1Stepper Driver Board
ST13	Stepper Motor Control Signals to 8-Port Valve Stepper Driver Board
ST18	Stepper Motor Control Signals to Pump 3 Stepper Driver Board
ST21	Stepper Motor Control Signals to Pump 2 Stepper Driver Board
ST23	Stepper Motor Control Signals to Pump 1 Stepper Driver Board
ST14	Monitor to RS232 between µC on Control Board and the Ethernet Module
ST16	RS232 Debug I/F to µC
ST17	Leak Sensor 1
ST18	Leak Sensor 2

Jumpers

Boot	When populated causes the Control Board to start in Boot mode after a reset.
Boot Ethernet	When populated causes the Ethernet Module to start in Boot mode after a reset. This is a special mode needed only if the application program on the ethernet module has somehow become corrupted such that the ethernet module does not respond and the standard download no longer works. See ***"Ethernet Module Bootloader" on page 57***
PGmode	When populated forces the control Board to immediately run in application mode. Control Board Boot Mode no longer operates.
W7	Links DGND to Chassis GND. (Populated)
W8	Do not use

Step-Motor Board Connector and Jumper Layout *5.3*

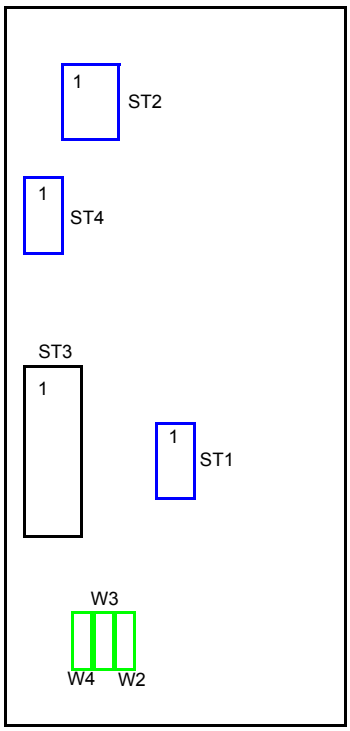


Figure 5.5. Step-Motor Driver Board Connections

ST2	Power + Rear Panel Fan Control on 8-Port Valve and EMAC control on Pump 1 - Pump 3 Stepper Driver Boards
ST4	Connector to Rear fan on 8-Port Valve and to EMAC on Pump 1 -Pump 3 Stepper Driver Boards
ST3	Stepper Motor Control Signals from Control Board
ST1	Stepper Motor Driver Signals to Stepper Motor.
W4..W2	Define the I2C address for the BIS EEProm on the Board Populated as Follows: (1 is populated, 0 is not populated, shown left to right)
Pump1	0 0 0
Pump2	0 0 1
Pump3	0 1 0
8-Port Valve	0 1 1

Wire Harnesses 5.4

Power Connector From Power Supply to Control Board 5.4.1

Table 5.1. Power Connector to Control Board

From	To	Color	Function
Power Supply V-	ST11 P1	blue	DGND
Power Supply V+	ST11 P2	red	+18V

Encoder Cable from Pump1 5.4.2

Table 5.2. Pump1 Encoder to Control Board (HZ13177)

From	To	Color	Function
Pump-1 Encoder P1	ST4 P1	brown	DGND
Pump-1 Encoder P3	ST4 P3	blue	Phase A
Pump-1 Encoder P4	ST4 P4	orange	+5V
Pump-1 Encoder P5	ST4 P5	yellow	Phase B

Encoder Cable from Pump 2 5.4.3

Table 5.3. Pump2 Encoder to Control Board (HZ13178)

From	To	Color	Function
Pump-2 Encoder P1	ST5 P1	brown	DGND
Pump-2 Encoder P3	ST5 P3	blue	Phase A
Pump-2 Encoder P4	ST5 P4	orange	+5V
Pump-2 Encoder P5	ST5 P5	yellow	Phase B

Encoder Cable from Pump 3 *5.4.4*

Table 5.4. Pump3 Encoder to Control Board (HZ13179)

From	To	Color	Function
Pump-3 Encoder P1	ST6 P1	brown	DGND
Pump-3 Encoder P3	ST6 P3	blue	Phase A
Pump-3 Encoder P4	ST6 P4	orange	+5V
Pump-3 Encoder P5	ST6 P5	yellow	Phase B

Cable from 8-Port Valve *5.4.5*

Table 5.5. 8-Port Valve Connector Cable (HZ13185)

From	To	Color	Function
	On Control Board		
8-Port Valve P10	ST7 P1	brown	DGND
8-Port Valve P9	ST7 P2	violet	Home Posn
8-Port Valve P8	ST7 P3	blue	Phase A
8-Port Valve P7	ST7 P4	orange	+5V
8-Port Valve P6	ST7 P5	yellow	Phase B
	On Stepper-Motor Board		
8-Port Valve P1	ST1 P1	red	Stepper Motor Phase A+
8-Port Valve P2	ST1 P2	red/yellow	Stepper Motor Phase A-
8-Port Valve P4	ST1 P4	green/black	Stepper Motor Phase B-
8-Port Valve P5	ST1 P3	green	Stepper Motor Phase B+

Home Sensor Cable from Pump 1 *5.4.6*

Table 5.6. Pump3 Encoder to Control Board (HZ13179)

From	To	Color	Function
Pump-1 Home Sensor	ST8 P1		DGND
Pump-1 Home Sensor	ST8 P2		Home Sensor
Pump-1 Home Sensor	ST8 P4		+5V

Home Sensor Cable from Pump 2 *5.4.7*

Table 5.7. Pump3 Encoder to Control Board (HZ13179)

From	To	Color	Function
Pump-2 Home Sensor	ST9 P1		DGND
Pump-2 Home Sensor	ST9 P2		Home Sensor
Pump-2 Home Sensor	ST9 P4		+5V

Home Sensor Cable from Pump 3 *5.4.8*

Table 5.8. Pump3 Encoder to Control Board (HZ13179)

From	To	Color	Function
Pump-3 Home Sensor	ST10 P1		DGND
Pump-3 Home Sensor	ST10 P2		Home Sensor
Pump-3 Home Sensor	ST10 P4		+5V

Power Cable from 8-Port Valve Stepmotor Board *5.4.9*

Table 5.9. 8-Port Valve Stepper Board to Control Board (HZ13176)

From (Stepper Board)	To (Control Board)	Color	Function
ST2 P1	ST12 P1	brown	+5V
ST2 P2	ST12 P2	red	ILIM1 (EMAC)
ST2 P3	ST12 P3	orange	DGND
ST2 P4	ST12 P4	yellow	ILIM0 (EMAC)
ST2 P5	ST12 P5	green	+18V
ST2 P6	ST12 P6	blue	/FAN_ENABLE
ST2 P7	ST12 P7	violet	PGND
ST2 P8	ST12 P8	white	FAN_SPEED

As the 8-Port Valve has no Electromagnetic Valve (EMAC), the valve driver on the stepmotor board is used to control the rear panel fan. The fan can run at 2 speeds: slow and fast.

Power Cable from Pump 3 Stepmotor Board *5.4.10*

Table 5.10. Pump 3 Stepper Board to Control Board (HZ13175)

From (Stepper Board)	To (Control Board)	Color	Function
ST2 P1	ST15 P1	brown	+5V
ST2 P2	ST15 P2	red	ILIM1 (EMAC)
ST2 P3	ST15 P3	orange	DGND
ST2 P4	ST15 P4	yellow	ILIM0 (EMAC)
ST2 P5	ST15 P5	green	+18V
ST2 P6	ST15 P6	blue	/EMAC_ENABLE
ST2 P7	ST15 P7	violet	PGND
ST2 P8	ST15 P8	white	EMAC_DIRN

Power Cable from Pump 2Stepmotor Board *5.4.11*

Table 5.11. Pump 2Stepper Board to Control Board (HZ13175)

From (Stepper Board)	To (Control Board)	Color	Function
ST2 P1	ST19 P1	brown	+5V
ST2 P2	ST19 P2	red	ILIM1 (EMAC)
ST2 P3	ST19 P3	orange	DGND
ST2 P4	ST19 P4	yellow	ILIM0 (EMAC)
ST2 P5	ST19 P5	green	+18V
ST2 P6	ST19 P6	blue	/EMAC_ENABLE
ST2 P7	ST19 P7	violet	PGND
ST2 P8	ST19 P8	white	EMAC_DIRN

Power Cable from Pump 1 Stepmotor Board *5.4.12*

Table 5.12. Pump 1 Stepper Board to Control Board (HZ13175)

From (Stepper Board)	To (Control Board)	Color	Function
ST2 P1	ST22 P1	brown	+5V
ST2 P2	ST22 P2	red	ILIM1 (EMAC)
ST2 P3	ST22 P3	orange	DGND
ST2 P4	ST22 P4	yellow	ILIM0 (EMAC)
ST2 P5	ST22 P5	green	+18V
ST2 P6	ST22 P6	blue	/EMAC_ENABLE
ST2 P7	ST22 P7	violet	PGND
ST2 P8	ST22 P8	white	EMAC_DIRN

Stepmotor Control Cable from 8-Port Valve Stepmotor Board *5.4.13*

Is a flatband cable connecting the Control Board ST13 to the Stepmotor Board ST3. This carries the control signals to the stepmotor driver ICs.

Stepmotor Control Cable from Pump 3 Stepmotor Board *5.4.14*

Is a flatband cable connecting the Control Board ST18 to the Stepmotor Board ST3. This carries the control signals to the stepmotor driver ICs.

Stepmotor Control Cable from Pump 2Stepmotor Board *5.4.15*

Is a flatband cable connecting the Control Board ST21 to the Stepmotor Board ST3. This carries the control signals to the stepmotor driver ICs.

Stepmotor Control Cable from Pump 1Stepmotor Board *5.4.16*

Is a flatband cable connecting the Control Board ST23 to the Stepmotor Board ST3. This carries the control signals to the stepmotor driver ICs.

Leak Sensor Connection *5.4.17*

Table 5.13. Leak Sensor to Control Board

From	To	Function
Leak Sensor	ST17 P1	+V Out (ca. 8V / 200K O/P Z)
Leak Sensor	ST17 P3	Hi Gain Amp. Input

Debug RS232 Connector on Rear Panel *5.4.18*

Table 5.14. Debug RS232 from Control Board (HZ13693)

From	To	Function
ST16 P1	Mini-D P2	RxD
ST16 P2	Mini-D P3	TxD
ST16 P3	Mini-D P5	DGND

Service 6

Degassing and Priming the Pumps 6.1

The Double Syringe Pump 6.1.1

Ensure that HyStar is not running as it might otherwise send unwished control commands to the BNMI-HP at this time!

Power up the unit and connect it to a PC either via an ethernet switch/hub or using a crossover cable. Check the tubing as shown in the diagram ***"BNMI-HP Tubing Connections Layout" on page 48***. Start your web browser and open 2 windows. In the first window select the Pump1 Test webpage (see ***"Low Level Operation: pump1.html" on page 21***). Click on 'Emergency Stop' to ensure that all pump and valve operations are killed.

On the 2nd window select the Main Functions web page (see ***"Main Functions Page: service.html" on page 15***). Here, Initialize the 8-port valve and then click on 'Block Flow' to move the valve to a position such that the flow is blocked.

Return to browser window 1 and select the Pump2 Test page. Click on 'EMAC Close' to ensure that the non-return inlet valve on Pump2 is closed and then return to the Pump1 Test page.

In the solvent flask ensure that the tubing in the filter sits at the bottom of the filter. Use the tubing to move the filter vigorously to ensure that the filter housing is filled with solvent and not gas.

Remove the fitting and tubing from the Pump 1 input valve. Hold the fitting with the tubing firmly against the end of the syringe and slowly suck in with the syringe until (only) liquid appears. The path from the solvent flask to the Pump1 EMAC input valve is now clear of gas. Replace the fitting and tubing in the inlet valve. (see ***"Correct seating of Fittings" on page 49***).

Click on 'EMAC Close' to ensure that the non-return inlet valve on Pump1 is closed this time. Remove the fitting together with its tubing from the Pump2 inlet valve. Hold the fitting with the tubing firmly against the end of the syringe and slowly suck in with the syringe until (only) liquid appears. The path from the solvent flask to the Pump2 EMAC input valve is now clear of gas. Replace the fitting and tubing in the inlet valve.

Put an object under the front of the BNMI-HP unit to tip the unit backwards and hence set the outlets of the pumps to be up from the inlet.

Replace the connections to the Splitter 'Main Flow In' and 'High Flow Out' with a plug.

Figure 6.1. BNMI-HP Tubing Connections Layout

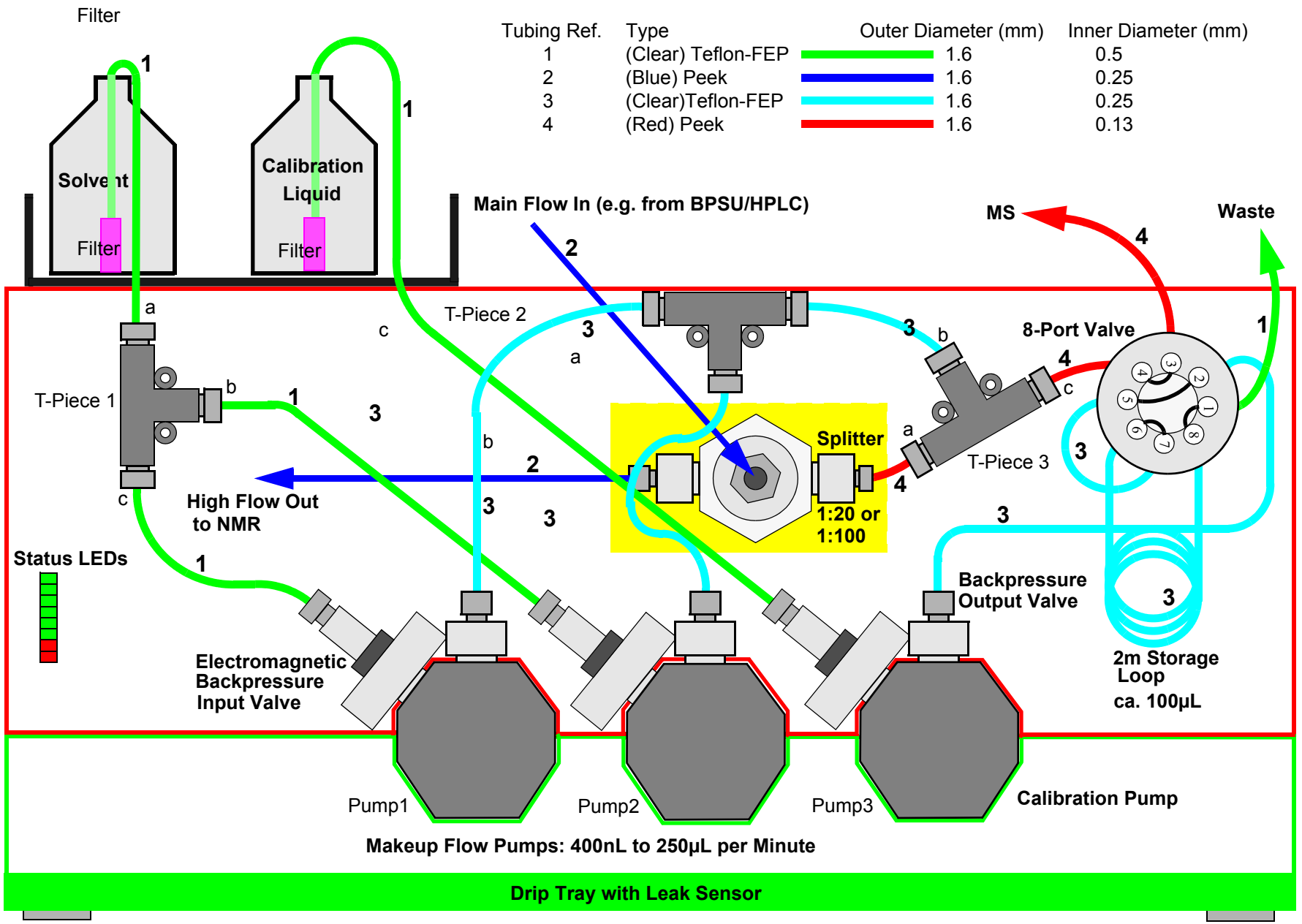
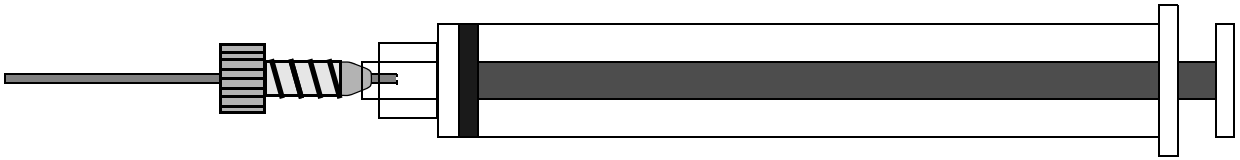
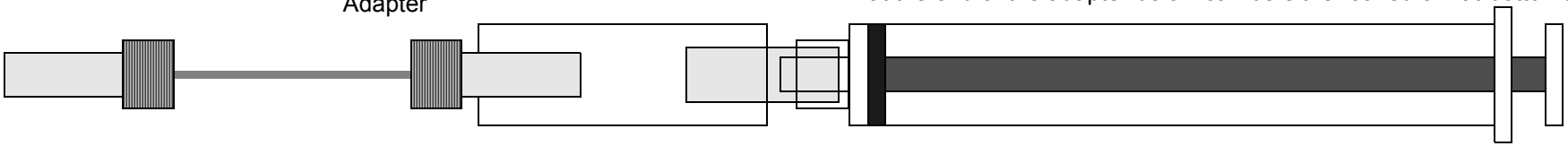


Figure 6.2. Degassing Tubing with a Syringe



Degassing length of tubing with
a 10-32 fitting at the end

Degassing a flowpath which terminates in a female port. The fitting at the end of the adapter below can be either coned or flat-bottomed.



Fingertight fitting for
coned port: 10-32 Thread

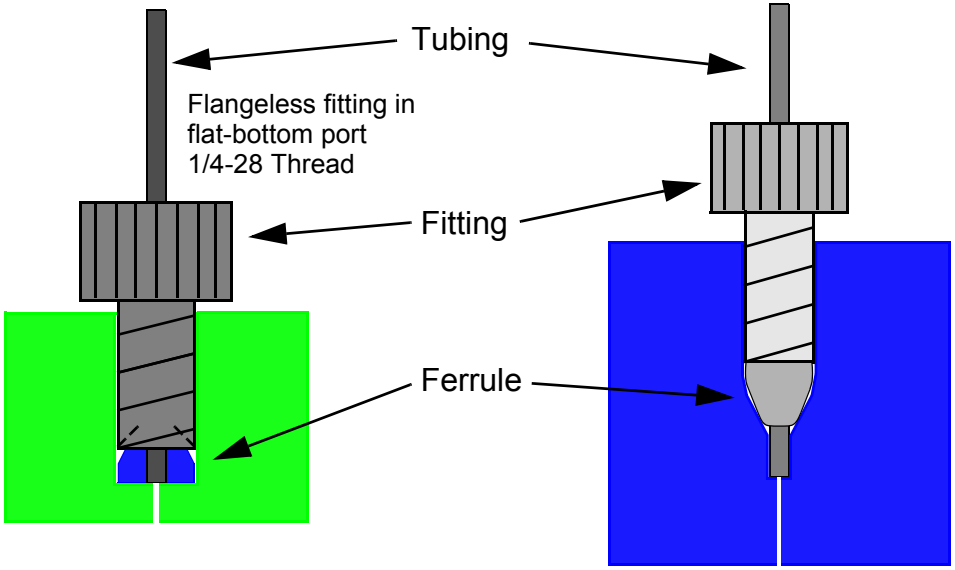


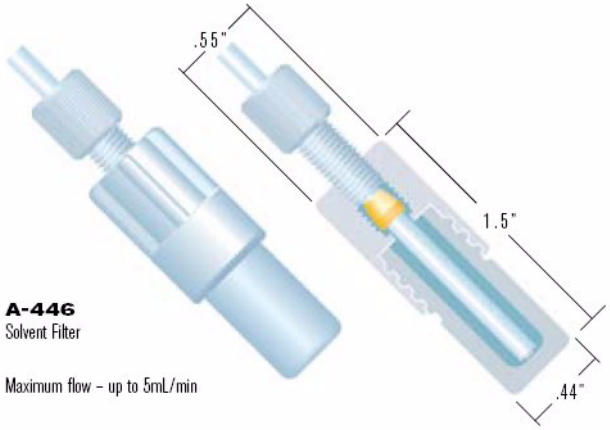
Figure 6.2. Correct seating of Fittings

In the BNMI-HP two types of fittings are used:

- **Flangeless Fitting for flat-bottom port (used only in the Pump outlet valves)**
- **Fingertight fitting for coned port (used everywhere else)**

*If you require to remove, change or alter any tubing connection you **must** ensure that the fitting is correctly seated.*
For a flat-bottom port the tubing should be flush with the bottom of the ferrule while screwing in the fitting. In the coned ports it should protrude 3-4 mm through the end of the ferrule. In both cases the ferrule should be free to rotate in the fitting (when screwing in a fitting which was previously in use, the ferrule is often jammed in the fitting. You should unjam it before screwing the fitting back into the port.). When you first feel resistance (this should be when the cone of the ferrule begins to be compressed), push the tubing into the fitting as you tighten the fitting further. This keeps the end of the tubing forced against the seat of the valve and ensures that no 'Dead Zone' occurs. Be especially careful not to overtighten fittings especially when using teflon tubing as you can inadvertently reduce, or even completely block, the internal diameter of the tubing at the clamp position.

Figure 6.3. Solvent Filter



The diagram to the left (taken from the Upchurch Catalog) shows how to correctly attach the tubing to the in-line bottle filter.

To degas only Pump1: Remove the fitting and tubing from the right hand outlet (b) of T-Piece 1 and replace with a plug. Loosen and remove the fitting to the inlet checkvalve at Pump1. With the piston of the syringe completely pushed in, hold this fitting against the end of the syringe as shown in ***page 49, Figure 6.2.***. Pull out the syringe plunger thereby sucking fluid through the filter into the syringe. This ensures that the tubing path to this fitting is degassed. Re-attach the tubing/ fitting to the Pump1 inlet valve.

Remove the outlet valve from Pump2. Attach the Luer adapter to

Replace the tubing/fitting from Pump 2 in the T-Piece. Remove the tubingOn the Pump1 Test webpage set the ’pump out’ flowrate to 250, ’pump in’ to 500, the 4 delays to 100 and click on ’Enable Encoder Check’. Finally click on ’Emac Auto’ and ’Start Pump1’. Until the pump has first found its ’Home Sensor’ its ’pump out’ flowrate is limited to 50µL/Min. Slowly pull out the syringe plunger to suck the solvent and, of course and any gas in the flowpath.

If the syringe plunger is (almost) fully pulled out then stop the pump, remove the syringe from the Luer connection and empty it into the waste storage flask. You should do this with the tip of the syringe held higher than the base, so that once you have fully depressed the plunger the tip of the syringe is still filled with fluid. Re-attach the syringe to the purge valve and start the pump once again. Repeat this process (suck, stop pump, empty syringe, start pump) until no more gas bubbles come through. Stop the pump, remove the syringe and reconnect the tubing.

Degassing Pump2 is done in the same manner. Here you have to first remove the connection to the base (b) of T-Piece 2 and replace with a plug. Close the EMAC valve on Pump1 and use the Pump2 Test webpage to run Pump2.

Degassing both pumps at the same time means you do not first have to replace any connections with plugs. However you cannot be 100% certain that you have degassed both pumps. This is performed as when degassing Pump1 and Pump2 but with both pumps running at the same time and EMAC control set to ’Auto’ in both cases.

The Calibration Fluid Syringe Pump *6.2.1*

Ensure that HyStar is not running as it might otherwise send unwished control commands to the BNMI-HP at this time!

Power up the unit and connect it to a PC either via an ethernet switch/hub or using a crossover cable. Check the tubing as shown in the diagram ***"BNMI-HP Tubing Connections Layout" on page 48***. Start your web browser and open 2 windows. In the first window select the Pump3 Test webpage (see ***"Low Level Operation: pump1.html" on page 21***). Click on 'Emergency Stop' to ensure that all pump and valve operations are killed.

On the 2nd window select the Main Functions web page (see ***"Main Functions Page: service.html" on page 15***). Here, Initialize the 8-port valve and then click on 'Transfer' to move the valve to a position such that the flow from the calibration pump would go directly to waste.

In the solvent flask ensure that the tubing in the filter sits at the bottom of the filter. Use the tubing to move the filter vigorously to ensure that the filter housing is filled with solvent and not gas.

Remove the fitting and tubing from the Pump 3 input valve. Hold the fitting with the tubing firmly against the end of the syringe and slowly suck in with the syringe until (only) liquid appears. The path from the solvent flask to the Pump3 EMAC input valve is now clear of gas. Replace the fitting and tubing in the inlet valve. (see ***"Correct seating of Fittings" on page 49***).

Remove the fitting and tubing from Port 2 of the 8-Port valve. This comes from the Pump3 outlet valve. On the Pump3 Test webpage set the 'pump out' flowrate to 250, 'pump in' to 500, the 4 delays to 100 and click on 'Enable Encoder Check'. Finally click on 'Emac Auto' and 'Start Pump3'. Until the pump has first found its 'Home Sensor' its 'pump out' flowrate is limited to 50µL/Min. Hold the fitting with the tubing firmly against the end of the syringe and slowly pull out the syringe plunger to suck the solvent and, of course and any gas in the flowpath.

If the syringe plunger is (almost) fully pulled out then stop the pump, remove the syringe from the fitting and empty it into the waste storage flask. You should do this with the tip of the syringe held higher than the base, so that once you have fully depressed the plunger the tip of the syringe is still filled with fluid. Re-attach the syringe to the fitting and start the pump once again. Repeat this process (suck, stop pump, empty syringe, start pump) until no more gas bubbles come through. Stop the pump, remove the syringe and reconnect the tubing. Any object placed under the BNMI-HP to tip it backwards can now be removed.

Purging the Flowpaths *6.3*

When first installing the BNMP-HP or if you change to a different solvent you should purge (i.e. wash out) the flowpaths with the new solvent to ensure all traces of any previously used solvent have been completely cleaned out.

Degas the pumps first as described above. As when degassing Pump1 and Pump2 together attach the luer connector of the syringe to the purge valve and put the 8-Port valve in the 'Blocked' Position. Set up Pump1 and Pump2 as described for degassing and start both pumps. Set both pumps to run at full speed (250µL/min pump and 500µL/min suck) and suck additional fluid into the syringe. 2-3mL should be sufficient. Stop the pumps.

Similarly for the calibration pump, attach the syringe as described for degassing. Run the pump and suck through 2-3mL solvent. Stop the pump remove the syringe an reconnect the fittings.

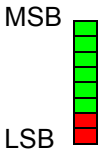
Set the 8-Port Valve to 'Transfer' Position (see ***"Operation of the 8-Port Valve" on page 17***). This sets the flowpaths such that the flow from the double syringe pump flows through both the short loop and the storage loop on the 8-Port valve and then out to the MS. The fluid from the calibration pump flows directly to waste.

Remove the connection to the MS and feed it into a waste container. Start Pump1 and Pump2 as above and/or Pump3. All 3 pumps can be purged at the same time if wished. Depending on the importance or otherwise of any contamination due to the previously used solvent, the pumps can be left to purge for a simply few minutes or even overnight if wished.

Do not start any of the pumps unless the outlet path is unblocked. e.g. to flow to waste, MS or into a syringe. Also ensure that the tubing in the solvent flasks sits at the bottom of the filter and that the bottom 5mm of the filter is completely submerged and filled with solvent.

Hints and Tips *6.4*

Status LEDs *6.4.1*



At the left hand side of the unit are 8 status LEDs. These have the following meaning.

For the following descriptions the top LED (green) is the Most Significant Bit and the Bottom LED (red) the Least Significant bit. A 1 means the LED is lit and a 0 means it is not lit. e.g. 1111000 means the top 4 green LEDs are lit.

During the power up or reset phase

The Control Board always starts in its *Boot* mode. At the start of the *Boot* mode the program checks:

1. Has the program been switched here from application mode? (i.e. during control board program download)
2. Is the *Boot* Jumper populated?
3. Can no valid *Application* program be found?

If any of 1-3 return a YES then the Control Board stays in Boot Mode

Start of Boot Mode: The Status LEDs show in sequence 0000011, then 0000000 to 11111100 counting through only the top 6 (green) LEDs. Then sets the LEDs to 10000000.

1 above is YES: Displays 11010000 and stays in *Boot* mode waiting for next command.

1 above is NO: Displays 11000000 and continues with checks 2 and 3.

2 above is YES: Still displays 11000000 and stays in *Boot* mode waiting for next command.

2 above is No: Still displays 11000000 and continues with check 3.

3 above is YES: Displays 11100000 and stays in *Boot* mode waiting for next command.

3 above is No: Still displays 11110000 and switches to Application Mode. (Normal Startup)

In Application Mode: The Status LEDs show in sequence 00000001, 00000010 ..... 10000000 (i.e. each LED lit in turn) to indicate that the application mode has started. This is followed by 00000100, 00001100 .... 11111100 (very fast) with each LED indicating that a board BIS data is being read. Finally it finishes on 10000000 indicating that the control board has finished its startup test and is running normally. Some 20-30 secs later this changes to 11000000 indicating that the ethernet module is now up and running.

i.e. in application mode, once the startup sequence is done, the top green LED is used as a 'Power Up' LED. The 2nd from Top LED indicates if the Ethernet module is communicating with the Control Board µC. The normal '**ready**' LED status with nothing running and no errors is 11000000.

During operation the LEDs have the following meaning:

Bit 5 11**X**xxxxx: **X** Blinks while Pump1 is pumping out. (Faster blinkrate <=> faster flowrate)
or lights continuously while Pump1 is sucking in.
Is not lit when the pump is stopped.

Bit 4 11x**X**xxxx: **X** Blinks while Pump2 is pumping out. (Faster blinkrate <=> faster flowrate)
or lights continuously while Pump2 is sucking in.
Is not lit when the pump is stopped.

Bit 3 11xx**X**xxx: **X** Blinks while Pump3 is pumping out. (Faster blinkrate <=> faster flowrate)

	or lights continuously while Pump3 is sucking in. Is not lit when the pump is stopped.
Bit 2 11xxx**X**xx:	**X** Blinks while the 8-Port Valve is moving otherwise the LED is not lit
Bit 1 1xxxxx**X**x	**X** Blinks (red) if an error has occurred otherwise the LED is not lit
Bit 0 1xxxxxx**X**	**X** Blinks (red) if a leak has occurred (leak monitor can be disabled in software) the LED goes out only once the leaking fluid is removed from the leaksensor. A leak is only indicated after the leak condition has been observed for > 20Secs.

In Boot Mode	During a download the LEDs have the following meaning.
11000000	Switched from application mode, waiting for next Command (during download).
11100000	Has not found a valid application program, waiting for next Command.
11001100	During erase flash routine (takes several seconds)
00000011	If an error occurs during the erase flash routine
11111100	The erase flash routine was done OK
XXXXXxxx	**XXXXX** counts from 00000 through 11111 during the flash program routine
	Additionally
Bit 0 xxxxxxx**1**	Is lit (red) if a programming error occurs
Bit 1 xxxxxx**1**x	Is lit (red) if an RS232 Com. error occurs
Bit 2 xxxxx**X**xx	X toggles on each valid RS232 command string.

Manual Download 6.5

Normally a download (firmware upgrade) is made using HyStar. However, if for some reason this is not possible you can also perform a manual download.

Control Board Download 6.5.1

Using an FTP program of your choice open a connection to the BNMI-HP using the following login details:

IP-Address	192.168.254.42 (is the default)
username	“bnmi“
password	“bnmi“

copy the control board firmware from the PC to the BNMI-HP (bnmixxxxxxx.hex or *.h86 where the release date in the form yyyymmdd is in the string xxxxxxx)

Close the FTP connection to start the download. You can follow the progress of the download by watching the LEDs or by starting a browser and refreshing the page 192.168.254.42/status.xml

At the start of the download operation the Flash is erased. If you refresh the status page while this is still in progress you get the webpage as shown in ***Figure 6.4.***

Once the erase has finished successfully and the program download is in progress a refresh displays the webpage shown in ***Figure 6.5.*** The value <DOWN> shows the % of the program already written to flash.

After the entire program has been successfully written to flash the webpage shown in ***Figure 6.6.*** will be displayed. In order for the BNMI-HP to correctly continue this webpage **must** first be displayed. Otherwise the Ethernet module simply waits at the end of its control board downloader routine

Figure 6.4. Flash Erase

Figure 6.5. Flash Write

Figure 6.6. Flash Write Complete

Ethernet Module Download 6.6

As in the Control Board download, using an FTP program of you choice open a connection to the BNMI-HP using the following login details:

IP-Address	192.168.254.42 (is the default)
username	“login“
password	“pw“

copy the ethernet module firmware from the PC to the BNMI-HP (bnmi_xxxxxxx_image.bin where the release date in the form yyyymmdd is in the string xxxxxxx)

Close the FTP connection to start the download. You can follow the progress of the download by watching the LEDs or by starting a browser and refreshing the page 192.168.254.42/status.xml.

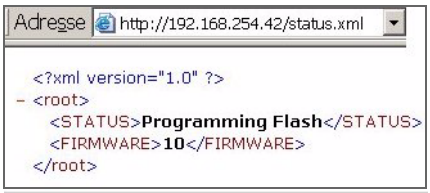
LED Status During Ethernet Module Download 6.6.1

At the start of the download all operations are killed. During the download the LEDs blink (approx. 2Hz) between 00101000 and 01010100.
NOTE: the LEDs only respond during an Ethernet Module Download if the Control Board is running in application mode. The download itself is otherwise unaffected by the control board firmware.

The download itself can take up to 30Seconds. Once it is over the Control board is (soft) reset and the Ethernet module is also reset.

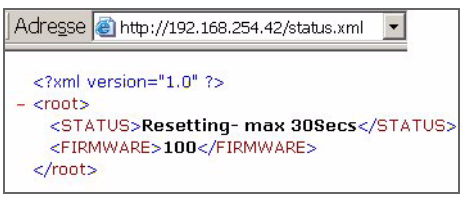
The Status LEDs show in sequence 00000001, 00000010 ..... 10000000 twice (i.e. each LED lit in turn) finishing on 10000000 indicating that the control board is running in application mode. Some 20-30 secs later this changes to 11000000 indicating that the ethernet controller is now up and running.

Status Webpage Display During Download 6.6.2



If you refresh the status page while the download is still in progress you get the webpage as shown on ***Figure 6.7.*** to the left.

Figure 6.7. Ethernet Module Flash Write



After the entire program has been successfully written to flash, the webpage shown in ***Figure 6.8.*** to the left will be displayed for a few seconds after which the Ethernet module sends a soft reset to the Control Board then resets itself.
The unit then requires about 30secs until it is fully operational again.

Figure 6.8. Ethernet Module Download Over

Ethernet Module Bootloader *6.7*

In the event that the application program in the ethernet module becomes corrupted (e.g. If the unit is powered down during a normal ethernet module download) then the module will no longer respond and a standard program update (download) is not possible.

Also if a valid program was downloaded but it does not have download support for future program updates then the previously described ethernet module download cannot be used. In this case we have to resort to using the bootloader routine.

Bootloader *6.7.1*

Ensure that the only units connected to the ethernet switch are the PC and the BNMI-HP: Either remove the network cables from the switch or power down the other units.

On the PC you need to have a TFTP server with DHCP. The freeware program **'TFTPD32'** available at http://tftpd32.jounin.net/ works very well. Check the 'help'.

Configure the DHCP server to allocate only the standard BNMI-HP IP-Address (192.168.254.42). Copy the BNMI_xxxxxxxx_image.bin file into the same directory as the tftp executable. Configure the tftp such that it will attempt to upload the this BNMI_xxxxxxxx_image.bin.

On the BNMI-HP remove the top (blue) cover. (**see Warning below**)

Populate the 'BOOT ETHERNET' jumper (or otherwise short circuit the 2 pins) (See the Control Board Layout: ***page 38, Figure 5.4.***)

The bootloader program has no influence over the Status LEDs. However after a successful download the 2nd Top green LED lights once this newly loaded application program is up and running.

If there is no sign of any activity then power cycle the ethernet switch and repeat the power cycle of the BNMI-HP with the jumper still populated (short circuited).

The bootload program is allocated the standard BNMI-HP IP address by the DHCP server and then the tftp server uploads the file BNMI_xxxxxxxx_image.bin. Some 30 seconds later the program will have been written to the Flash memory and the BNMI-HP will restart. Remove the jumper and replace the top cover.

This method is only required if some disaster has happened. Be sure you understand how to set up the DHCP and TFTP servers before attempting this method.

The BNMI-HP cover should only be removed by a technically competent person and with the power cable removed. Once you have populated the jumper, replace the cover before powering up the unit.

The Debug Interface *6.8*

At the rear of the BNMI-HP unit is a 9 pin male mini-d RS232 connector. This is the debug interface. See:
"BMNI-HP Rear View showing Electrical Connections" on page 7
and
"Debug RS232 Connector on Rear Panel" on page 46.

This interface is intended only for setup and debug purposes and is not used during normal operation. To connect to a PC for use with a terminal program (e.g. Hyperterminal) you need a 9pin Female to 9 Pin Female X-over cable.

Default RS232 Configuration: 19200 Baud, 8 Data Bits, 2 Stop Bits, No Parity.

The ***only*** use of this interface outwith the test environment is to allow the IP address to be changed, or interogated in the event that it had been changed from the default value and forgotten!)

Connect the debug connector to a PC and start your terminal program. Configure the terminal program as described above. Enable the local echo (half-duplex) so that what you type in the keyboard is displayed immediately.

Power up the BNMI-HP and wait till the status LEDs display 10000000 indicating that the control board is up and running: this takes ca. 2-3 secs.

To check that the interface is connected and configured correctly you can enter one of the test commands finishing with the Carriage Return (Enter) key.

e.g.

You enter

AV

and the unit should reply with the Application Firmware Version

AV060123-14:19

If the unit does not respond then you have some connection or configuration problem.

To Change or Interrogate the IP Address *6.8.1*

This can only be done while the Ethernet Module is starting up, i.e. initiated within the first 15-20Secs after reset or power up.

With the unit connected as above and the debug interface operational, turn off the local echo in the terminal program (= full duplex mode) then reset or power cycle the unit. As soon as the status LEDs show 10000000 enter the command:

DB1 (NOTE: no local echo so not visible)

and the unit responds with:

DBOKLoop through to ConnectME enabled

This sets the debug interface into 'Loop Through' mode. This means that serial data coming from the Ethernet Module to the control board is additionally looped through to the debug interface. Also characters entered via the debug interface are sent on to the ethernet module.

Assuming you have managed to enter the command before the ethernet module had finished its initialisation sequence, sometime in the next 10-20secs the terminal will display:

```
NET+WORKS Version 6.2
Copyright (c) 2000-2004, NETsilicon, Inc.
PLATFORM: connectme
APPLICATION: BNMI-HP Application Program
------------------------------------------------------------------------------
NETWORK INTERFACE PARAMETERS:
IP address on LAN is 192.168.254.42
LAN interface's subnet mask is 255.255.255.0
IP address of default gateway to other networks is 0.0.0.0
HARDWARE PARAMETERS:
Serial channels will use a baud rate of 19200
This board's serial number is A00000016
This board's MAC Address is 00:40:9D:25:BE:D1
After board is reset, start-up code will wait 3 seconds
Default duplex setting for Ethernet connection: phy Default
------------------------------------------------------------------------------
Press any key in 3 seconds to change these settings.
```

Hit the **'Enter'** or any other key within 3secs and the following is displayed:

```
Press A to Accept the settings, or M to Modify?
```

Enter **m** to allow you to modify the settings. The unit responds with:

```
Enter the root password:
```

Enter **sys** (the root password). The unit responds with:

```
Reset configuration to default values (Y/N)?
```

Which we do not want to do so simply hit the 'Enter' key:

```
For each of the following questions, you can press <Return> to select the value shown
in braces, or you can enter a new value.
NETWORK INTERFACE PARAMETERS:
Should this target obtain IP settings from the network?[N]
```

Normally we work with a fixed IP address, however if the network has a DHCP server and your control program can work with dynamically allocated IP addresses then you can enter **y** otherwise hit the '**Enter**' key:

```
Static IP address [192.168.254.42]?
```

Here you can either accept the IP address by hitting the '**Enter**' key or you can enter a new address in exactly the same format. (i.e. **aaa.bbb.ccc.xxx**).

NOTE: The IP address of the control PC must have the aaa, bbb and ccc components the same as you enter here otherise the ethernet communication will not function. Also if you change the IP-address and the unit was connected via an ethernet switch you may have to power cycle the switch to clear its MAC - IP table.

The unit continues as follows (here with the IP address unchanged)

```
Subnet Mask IP address [255.255.255.0]?
```

Change or accept ('**Enter**' key) as wished:

```
Gateway address IP address [0.0.0.0]?
```

Is not used so hit the **'Enter'** key:

```
SECURITY PARAMETERS:
Would you like to update the Root Password?[N]
```

Do NOT change! Hit the '**Enter**' key.

Would you like to update the Administrator 'sys' Password?[N]

Do NOT change! Hit the '**Enter**' key.

HARDWARE PARAMETERS:
Set the baud rate of Serial channels[19200]?

Do NOT change! Hit the '**Enter**' key.

The new baud rate is 19200
The baud rate will be changed on next power up
Please set the baud rate for your terminal accordingly
Each development board must have a unique serial number
Set the board's serial number[A00000016]?

Do NOT change! Hit the '**Enter**' key.

Each development board must have a unique Ethernet MAC address.
Set the board's Ethernet MAC Address[00:40:9D:25:BE:D1]?

Do NOT change! Hit the '**Enter**' key.

How long (in seconds) should CPU delay before starting up[3]?

Do NOT change! Hit the '**Enter**' key.

Normally the board will automatically negotiate with the network hub

etc. until

Default Ethernet duplex setting (Full, Half, or phy Default)?[phy Default]

Do NOT change! Hit the '**Enter**' key.

Saving the changes in NV memory...Done.

The unit saves the new parameters and performs a soft reset. A few seconds later the original startup message is displayed agan:

NET+WORKS Version 6.2

etc until....

Press any key in 3 seconds to change these settings.

This time do NOT press any keys (unless you made a mistake earlier and wish to change the IP address again !). After 15-30 secs the following is displayed:

ACE: Have IP address on interface eth0: 192.168.254.42
Network IP configured.
Begin the NAHTTP Server application.
FTP Downloader Ready: user/password =bnmi/bnmi
NAFTPSVR Application Ready.

The unit is now running with the IP-Address shown above. The loop through mode will have been automatically disabled again.

If you change anything other than the IP-Address and the subnet mask then the unit may not operate correctly. In particular Do NOT change the baudrate. The Loop-through only supports 19200 and 9600 baud so if you set it to anything else you will not be able to restore working values again!

The 8-Port Valve Positions *6.8.2*

Command Required

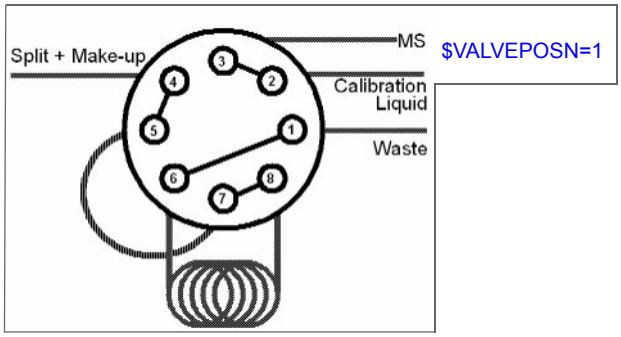
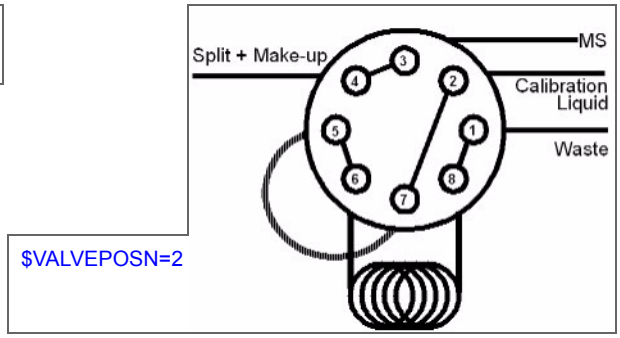


Figure 6.9. Position 1 'undefined'



Position 2 'undefined'

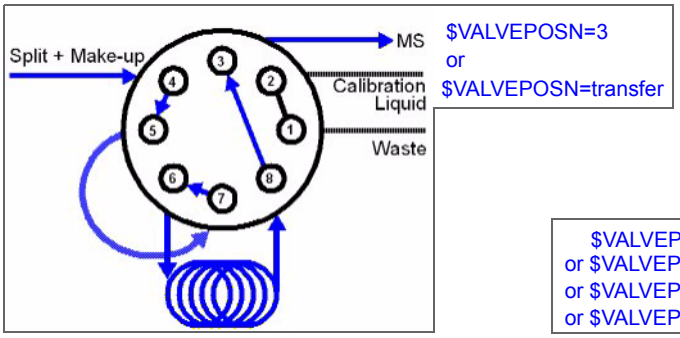
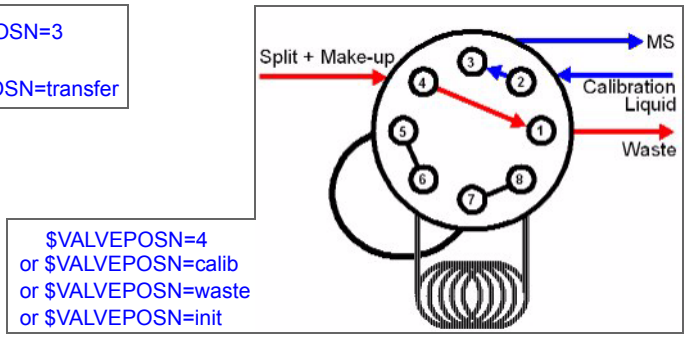


Figure 6.10. Position 3 'transfer'



Position 4 'calib' or 'waste' or 'init'

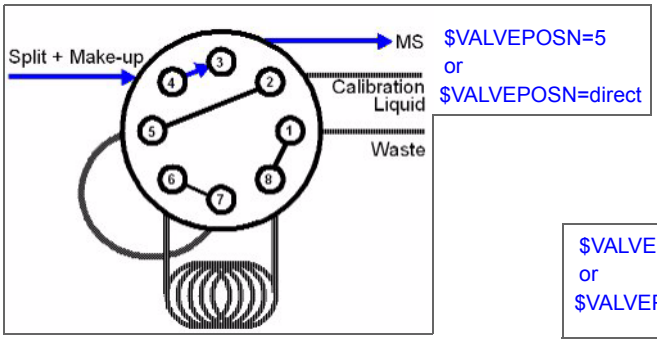
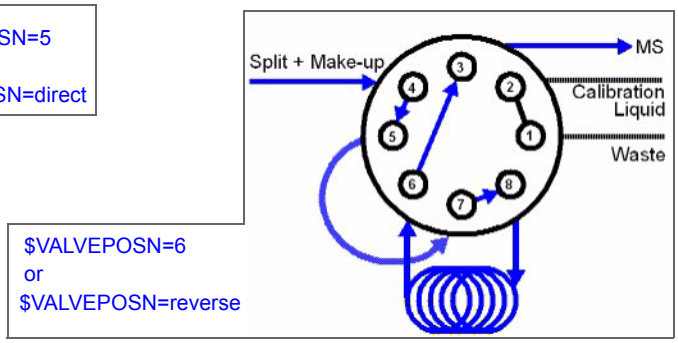


Figure 6.11. Position 5 'direct'



Position 6 'reverse'

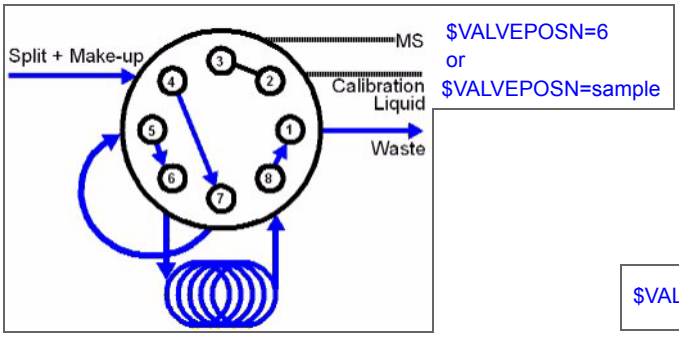
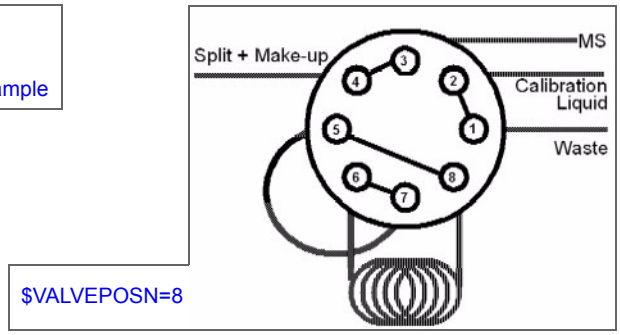


Figure 6.12. Position 7 'sample'



Position 8 'undefined'

Gradient Pump Operation *6.9*

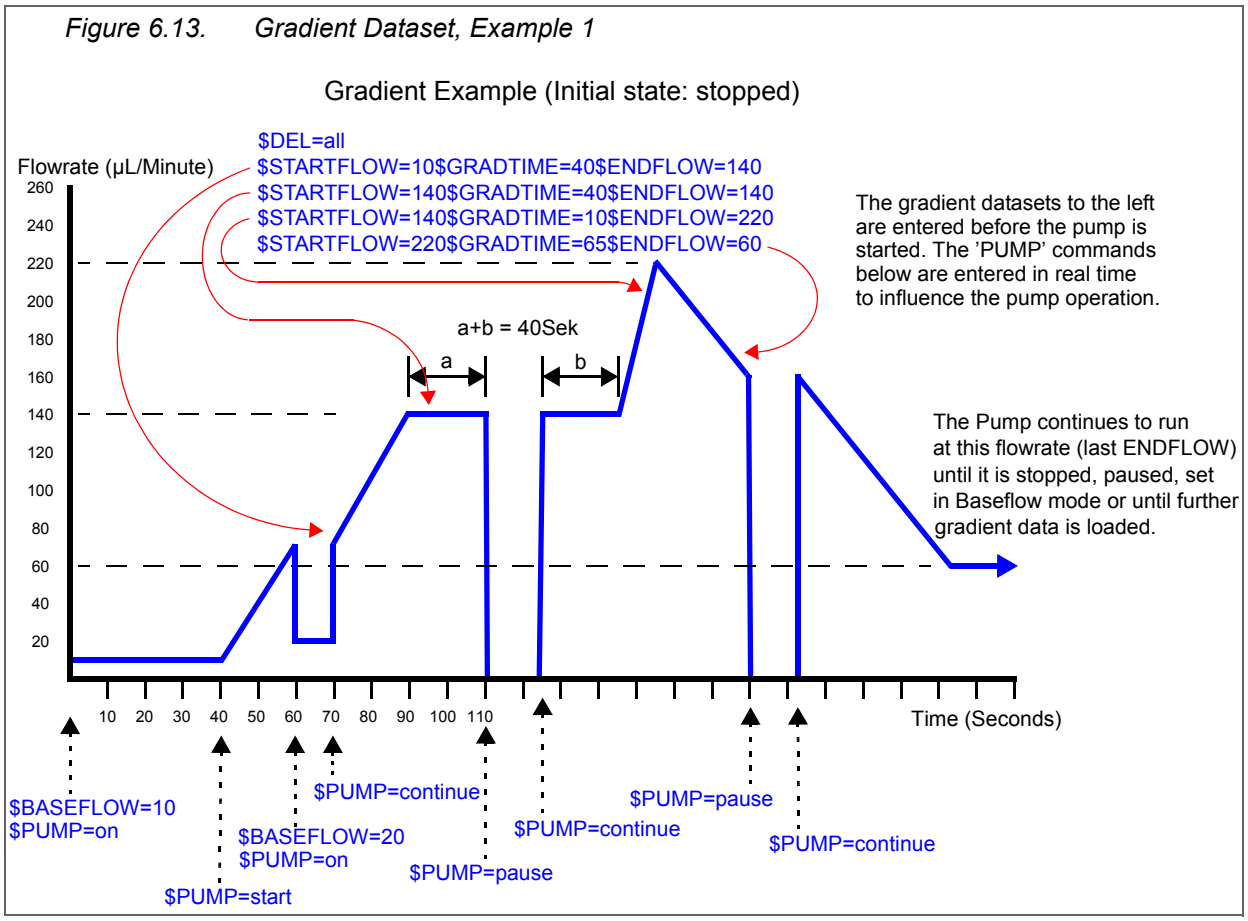
A (flow) gradient in the BNMI-HP consists of 3 parts:

Startflow When implementing a gradient dataset this is the initial flowrate. (in µL/Min)

Endflow This is the final flowrate (in µL/Min).

Gradient Time and this is the time taken for the flowrate to change linearly from the Startflow to the Endflow. (in seconds)

Figure 6.13. Gradient Dataset, Example 1



When entering a gradient dataset the Endflow value must be entered last. On receiving the Endflow value the 3 components of the dataset are saved to the gradient table. If no value for the Startflow and the Gradient time have been entered these are replaced with 0.

When the $PUMP=start command is sent the pump will only start if at least one data set is available. If a Startflow had been defined the pump goes immediately to this flowrate (a step change is not actually possible but the acceleration is fast enough that you can treat it as such). If Startflow=0 and the Pump is in Baseflow mode then the pump takes the baseflow flowrate to be its start flowrate.

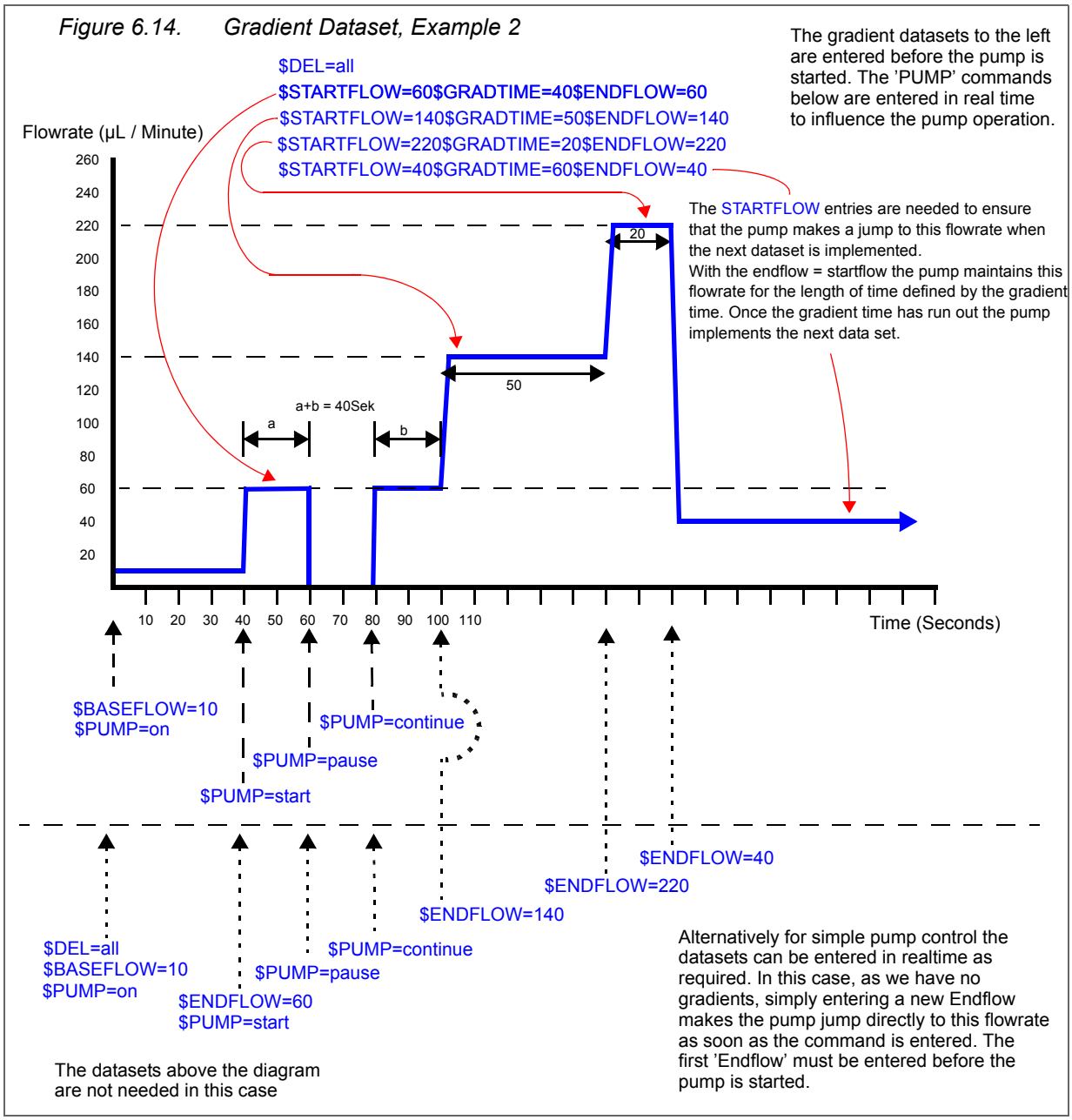
The $PUMP=on command causes the pump to immediately pump at the predefined baseflow flowrate. The present flowrate and remaining gradient time are saved. The $PUMP=continue command makes the pump restart at the same point in the pump gradient where it had been when switched to baseflow mode.

The $PUMP=pause command works similarly but here the pump is completely stopped.

Once the gradient time has run out then the next dataset from the gradient table is implemented. If no Startflow is defined for this data set (i.e. is = 0) then the Endflow (= present flowrate) from the previous dataset is used as the Startflow.

If no further gradient data is available the pump continues to pump with the last Endflow flowrate. If new gradient data is later entered, it is implemented at once.

Figure 6.14. Gradient Dataset, Example 2



Example 2, above shows how to implement a simple step change of flowrate. Here, there are 2 ways to achieve the same result:

1. Enter the data sets then start the pump.
2. Enter the first dataset. Start the pump then enter the new datasets as and when they are required. As the gradient time (= 0 here) has always run out, the gradient corresponding to the data set is implemented at once.

Calibration Pump Operation *6.10*

The purpose of the calibration pump is to inject a small volume of an easily identifiable (in the MS spectrum) fluid into the sample flowpath to the MS to enable the start and end (and any other key times) of an experiment to be easily identified.

As the volume normally dosed is in the range 5.0µL to 95.0µL, this can be done within one cycle of the calibration pump. However for washing purposes the maximum dose volume is 6500.0µL and a demand volume = 0 enables the pump to run endlessly until stopped.

The demand pump flowrate is set with the command $CALIBFLOW=xxx where xxx can be 0.4 to 250 (µL/minute).

The demand dose volume is set with the command $CALIBDOSE=xxx where xxx can be 0.1 to 6500.0 or 0 (µL).

The Flowrate and Dose volume should be set before starting the pump. Also the initialisation routine should be run before starting the pump. Also the 8-Port valve should be initialized as it is moved during the calibration operation.

When a calibration pump cycle is started ($CALIBPUMP=start) the unit first saves the present position of the 8-Port valve then moves the valve to the 'calib' position. Here the flowpaths are: calibration pump to MS and makeup flow+split is fed to waste. As soon as the valve has switched, the pump pumps at the predefined flowrate until the predefined volume has been dosed. The 8-Port valve is then switched back to the position where it had been before the calibration pump operation had been started, and the <RUN> status is set to **end** to flag the end of the calibration cycle.

At this point the calibration pump reverses direction and sucks calibration fluid ready for the next calibration cycle.

If a $CALIBPUMP=start command is received before the pump has finished sucking fluid in, as long as sufficient fluid is available to dose the demand volume in one pump cycle, the pump immediately stops sucking and starts the calibration pump operation. If there is insufficient fluid available then the pump continues to suck and the <RUN> status is set to **busy.** This can only really happen if the time between a calibration cycle ending and the next one beginning is:

< 12 * (dose volume)/100 seconds.

e.g. for a demand calibration dose volume of 50µL you should leave at least 6 seconds after the operation has ended before starting another cycle.

Physical Specifications *6.11*

BNMI-HP

Height (Chassis)	165mm
Height (Chassis + Flask Storage)	215mm
Width	320mm
Depth	200mm
Weight (without solvent flasks)	10kg
Power Requirements	115 - 230V 50 - 60Hz 100VA
Fuses	2 x 0.8A, 250V

Operational Environment

10°C to 30°C Non Condensing Air Humidity

The main earth connection to the Unit is supplied via the largest pin in the Euro-Standard 3 Pin connector and must be connected to ground using either the mains cable supplied or one of similar specifications. Incorrect earthing of the unit can be very dangerous.

Figures

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info@bruker-biospin.com
www.bruker-biospin.com