Agilent 1290 Infinity LC Injectors HTC/HTS

User Manual

Agilent Technologies

Notices

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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

In This Book

This manual covers the Agilent 1290 Infinity LC Injectors HTC/HTS

1 General Information

This chapter provides safety information and gives an introduction on how to use this manual.

2 Operating Instructions

This chapter explains the operational parameters of the Agilent 1290 Infinity LC Injectors HTC/HTS System.

3 Description and Installation

This chapter gives an overview and information about the installation of your Agilent 1290 Infinity LC Injectors HTC/HTS.

4 Appendices

This chapter provides addition information.

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1 General Information

This chapter provides safety information and gives an introduction on how to use this manual.

Safety Information

General Considerations

The LC Injector HTC/HTS System User Manual and the corresponding “Addendum” for a specific module must be consulted by the user under all circumstances before a unit is put in use.

Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

When using the LC Injector HTC/HTS System, follow the generally accepted procedures for quality control and methods development.

When you use the LC Injector HTC/HTS System in the field of chromatographic analysis and you observe a change in the retention of a particular compound, in the resolution between two compounds, or in peak shape, immediately determine the reason for the changes. Until you determine the cause of a change, do not rely on the separation results.

Electrical Hazards

Every analytical instrument has specific hazards, so be sure to read and comply with the following precautions. They will help ensure the safe, long-term use of your LC Injector HTC/HTS System.

The Installation Category (Over voltage Category) for this instrument is Level II. The Level II Category pertains to equipment that receives its electrical power from the local level, such as an electrical wall outlet.

Only use fuses of the type and current rating specified. Do not use repaired fuses and do not short-circuit the fuse holder.

The supplied power cord must be inserted into a power outlet with a protective earth contact (ground). When using an extension cord, make sure that the cord also has an earth contact.

Do not change the external or internal grounding connections. Tampering with or disconnecting these connections could endanger you and/or damage the LC Injector HTC/HTS System.

The instrument is properly grounded in accordance with these regulations when shipped. You do not need to make any changes to the electrical connections or the instrument's chassis to ensure safe operation.

The combination of a LC Injector HTC/HTS System with a LC/MS System does require the safety measure as described by the LC/MS System manufacturer. Detailed instructions for the safety grounding on the LC/MS system are outlined in the corresponding operating/installation manual.

Agilent Technologies recommends to use a grounding cable connected on one side at the Injection Valve, Loop or any other suitable direct metallic contact and the other side at an appropriate grounding point at the LC/MS System. This supplementary grounding measure will support the safety strategy of the LC/MS System manufacturer.

Do not turn the instrument on if you suspect that it has incurred any kind of electrical damage. Instead disconnect the power cord and contact a Agilent Technologies representative for a product evaluation. Do not attempt to use the instrument until it has been evaluated. Electrical damage may have occurred if the LC Injector HTC/HTS System shows visible signs of damage, exposure to any liquids or has been transported under severe stress.

Damage can also result if the instrument is stored for prolonged periods under unfavorable conditions (e.g. subjected to heat, water, etc.).

In any case disconnect the power cord(s) from the power supply or from the different power supplies if optional devices are installed before attempting any type of maintenance.

Capacitors inside the instrument may still be charged even if the instrument is turned off.

To avoid damaging electrical parts, do not disconnect an electrical assembly while power is applied to the LC Injector HTC/HTS system. Once the power is turned off, wait approximately 30 seconds before you disconnect an assembly.

The instrument includes a number of integrated circuits. These circuits may be damaged if exposed to excessive line voltage fluctuations and/or power surges.

Never try to repair or replace any components of the instrument that is not described in this manual without the assistance of a Agilent Technologies representative.

There are no operator-serviceable or replaceable parts inside the power supply(ies) or in the LC Injector HTC/HTS System. If a power supply is not functioning, contact a Agilent Technologies representative.

The power supply for the LC Injector HTC/HTS Instrument has the symbols 1/0 on the label for the power switch to switch ON/OFF.

Any additional power supply for other devices like, Cooled Stack or a Valve Module shows the symbols as shown below on the label for the power switch:

The symbols shall warn the user that in a emergency case more than one power supply has to be turned OFF or more than one power cord has to be pulled from power supply or from the wall outlet to shut down the complete LC Injector HTC/HTS System.

If the basic LC Injector HTC/HTS System is installed, than a single power supply is installed only. Turning OFF the power supply or pulling this single power cord in an emergency case will stop the complete LC Injector HTC/HTS System.

It is important that the power supply (ies) are in a location where the power ON and OFF switch is accessible and easy to operate, and where it is possible to unplug the AC power cord from the power supply/wall outlet in case of emergency.

Other Hazards

To avoid injury during LC Injector HTC/HTS System operation, keep your hands away from the syringe.

Do not operate the LC Injector HTC/HTS System without the safety shield. The safety shield must be installed for safe operation.

To avoid injury, observe safe laboratory practice when you handle solvents, change tubing, or operate the LC Injector HTC/HTS System. Know the physical and chemical properties of the solvents you use. See the Material Safety Sheets from the manufacturer for the solvents in use.

Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Do not use polymer tubing that has been severely stressed or kinked.
- Do not use polymer tubing, in particular not PEEK or Tefzel tubing, with Tetrahydrofuran (THF), Dimethylsulfoxid (DMSO), chlorinated organic solvents, concentrated mineral acids, such as Nitric, Phosphoric or Sulfuric acids, or any related compounds to above listings.

Do not use vials without a sealing cap, microtiter or deepwell plates without a plate seal. Vapor phase from organic solvents can be hazardous and flammable. Acidic vapor phase can cause corrosion to critical mechanical s.

Disposal

Do not dispose of this equipment or parts thereof unsorted in municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). European Union customers: Call your local customer service representative responsible for the PAL System for complimentary equipment pick-up and recycling.

Lithium battery

An onboard lithium battery buffers the electronic memories, when the instrument is turned off. Replace it only with the same or equivalent type recommended by the equipment manufacturer.

Battery: Panasonic VL 2330, soldered directly on the electronic board. Discharged lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

There are no operator-serviceable parts on the electronic boards. If an electronic board fails, contact a Agilent Technologies representative.

Table 1 Commonly Used Symbols

Symbol	Description
	Caution or refer to User Manual
	Caution, Risk of Needle-Stick Puncture
	Caution, Hot Surface or High Temperature
	Direct Current
	Alternating Current
	Protective Conductor Terminal, Ground
	Fuse
I	Electrical Power ON. Used with Main LC Injector HTC/HTS Power Supply.
O	Electrical Power OFF. Used with Main LC Injector HTC/HTS Power Supply.
	Electrical Power ON for Only Part of the System. Used with Optional Device(s)
	Electrical Power OFF for Only Part of the System. Used with Optional Device(s)
	Caution, Risk of Electrical shock (high voltage)
	Disposal, Do not dispose in municipal waste. Follow local waste regulations to reduce electrical and electronic waste (WEEE).

How to Use this Manual

The manual is divided into three major sections

- “Operating Instructions” on page 15)
- “Description and Installation” on page 45)
- “Appendices” on page 191

The "LC Injector HTC/HTS Operating instructions" are intended for infrequent LC Injector HTC/HTS users or new users that are experienced in using automated systems to perform existing analytical methods.

NOTE The LC Injector HTC/HTS must be installed and set up properly before the Operating Instructions in Section A can be used.

Users who are installing a LC Injector HTC/HTS system, LC Injector HTC/HTS accessories or who need to make adjustments to an installed system should consult "LC Injector HTC/HTS Description and Installation" in Section B.

The Appendices provide useful information such as the Software Flow Chart, Definition of Terms or the LC Injector HTC/HTS Accessories guide.

2
Operating Instructions

This chapter explains the operational parameters of the Agilent 1290 Infinity LC Injectors HTC/HTS System.

Using the Control Terminal

The following procedures present the key steps required to set up and process multiple groups of samples with the system. They are intended to provide an overview for new users and a reminder for infrequent users. The system and all accessories should be installed with Objects defined correctly. A syringe of the specific type called for by a particular method should also be installed.

Figure 1 on page 17 illustrates the system control terminal and the conventions used to enter, edit, and view information.

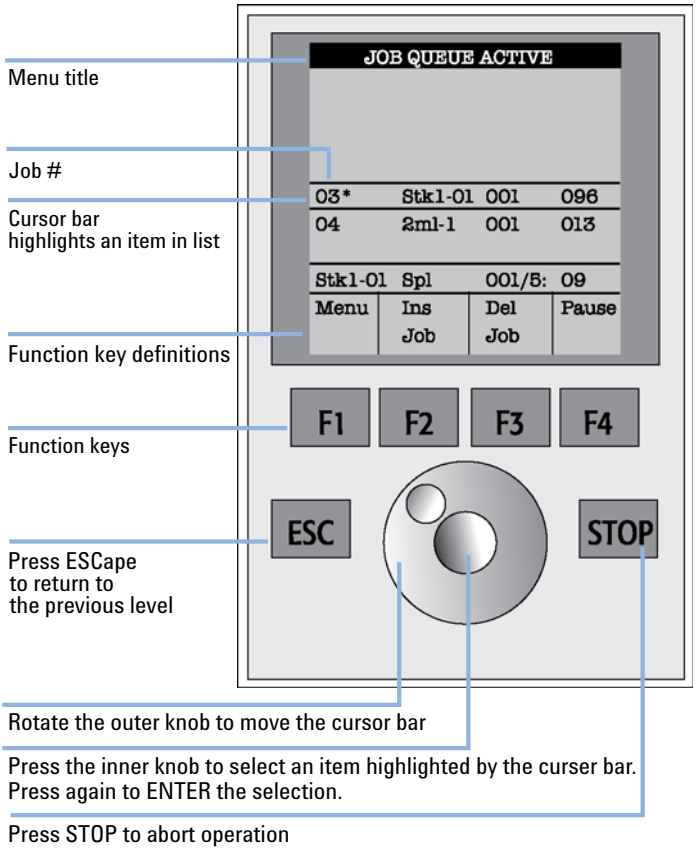


Figure 1 System Control Terminal and Conventions

Menu Screens

Different menu screens are displayed, depending on the system operating status and the particular function being accessed by the operator. All menu screens have the same basic format. The menu title is displayed at the top of the screen. A list of items is displayed below the title. The date and time, or status, are shown in the highlighted area above the Function key labels on the bottom of the screen.

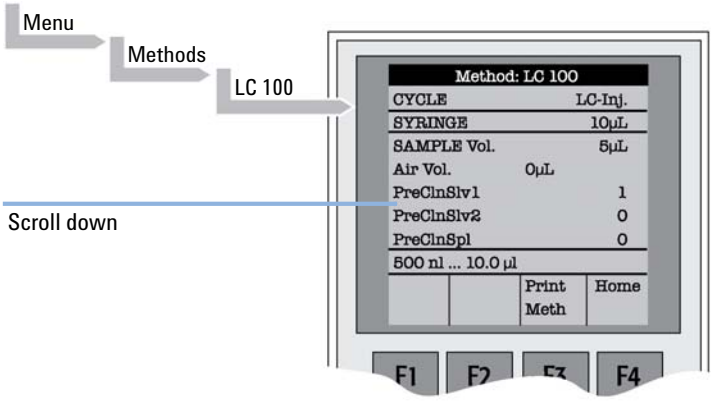


Figure 2 Accessing a Method Screen

Function Keys

Options for a particular menu are assigned to the corresponding function keys (**F1**, **F2**, **F3**, **F4**) directly below each function key label.

Pressing the function key labeled as **Home** will always return to the **Job Queue** menu.

ESCape and STOP Keys

Press **ESC**ape to return to the previous menu. Press **STOP** to abort the current **Cycle**, **Job**, or **Job Queue**.

Scroll Knob and ENTER Button

Rotate the outer knob to scroll through items in a menu list. To select a highlighted item press the central knob (**ENTER**). Then use the outer knob to scroll through available options for that item or to change a numeric value. Then press the inner knob again to *ENTER* the displayed option. The inner knob is also used for other operations that require an *ENTER* operation to continue or complete an operation.

Methods

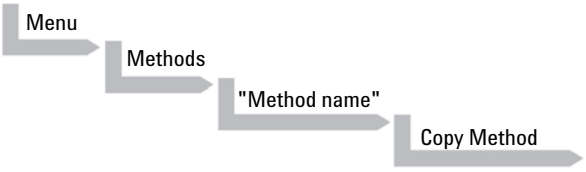
Creating Methods

Methods can be defined by the user and assigned names up to eight characters in length. **Methods** can be created, copied, edited, and viewed from the Methods menu. Methods can be viewed (but not edited) from the Job Queue menus.

Methods are created by either copying an existing Method or creating a new Method.

To copy a Method, complete the sequence as follows:

1

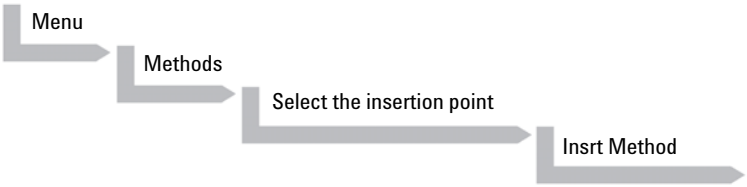


You will be prompted to enter a name for the new Method.

2 Use the scroll knob and the left-right arrow function keys (**F2** and **F3**) to select among alphanumeric characters and spaces. Press the **ENTER** function key (**F4**) to accept the name.

To create a new Method, complete the following steps:

1



2 Assign and enter a new Method name as above.

After a copy of the Method has been created, the Method parameters will display and can be edited. The Cycle and Syringe entries cannot be changed.

3 If the Method is new (i.e. added), select and enter a Cycle that is appropriate for the application.

4 Select the specific Syringe to be used by the Method.

NOTE Once a Method has been created and saved, the Cycle and Syringe cannot be changed. To use a different Cycle or Syringe, a new Method must be created.

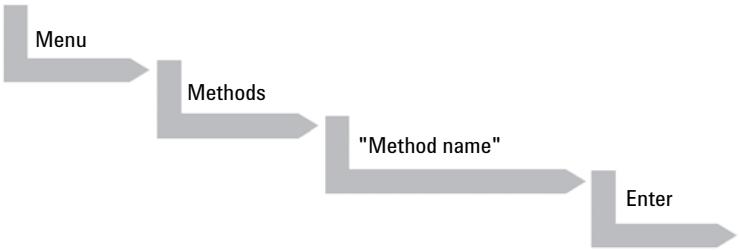
5 Assign Parameter values according to the application requirements. Consult ""LC-Inj" Cycle" on page 196 for details on specific items.

Edit / View Methods

Method parameters (excluding Cycle and Syringe) can be viewed and changed from the Method menu as follows:

Complete the following menu selections:

1



2 Scroll to and select the parameter to be changed. Assign the new value and press **ENTER**.

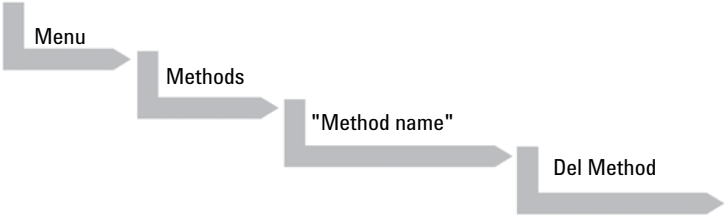
3 Exit from Parameter List by pressing either the **Home** function key (**F4**) to return to the top-level **Job Queue** menu or **ESC**ape to return to the previous menu.

4 Method contents may be viewed from the **Job Queue** displays by selecting the desired Job, pressing **ENTER**, followed by the **View Method** function key.

Delete Methods

Methods can be deleted from the Methods menu. Methods in use by an active Job cannot be deleted.

1 Complete the following menu selections to delete a Method.



Job and Job Queue

A **Job** bundles the specified Tray with the designated vials (samples) and with the Method to run those samples. Another term often used for “Job” in the chromatographic field is “sequence”. If more than one **Job** is prepared, the term **Job Queue** is used.

Before a Job can be activated the operator must verify that the Tray Type matches the specified Tray and vial size (type). This step is done in Utilities class.

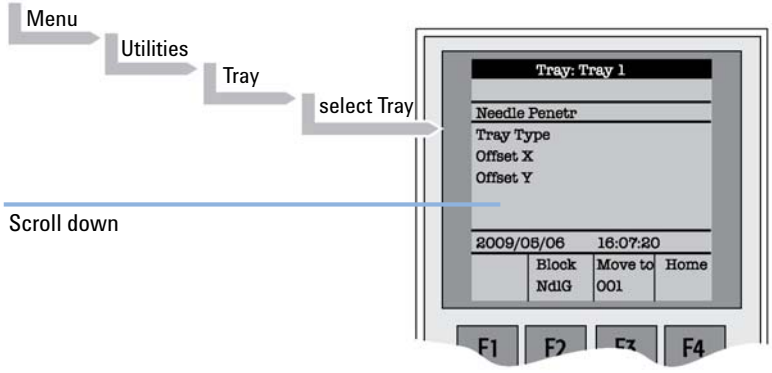


Figure 3 Verifying Tray with corresponding Tray Type

1 Select the corresponding Tray Type. By activating **F3** the injection unit can be moved to the first position and to another two corner positions to verify the correct selection of the Tray Type and teaching position.

Building and Starting a Job Queue

1 Power up the module. The **JOB QUEUE** screen is displayed.

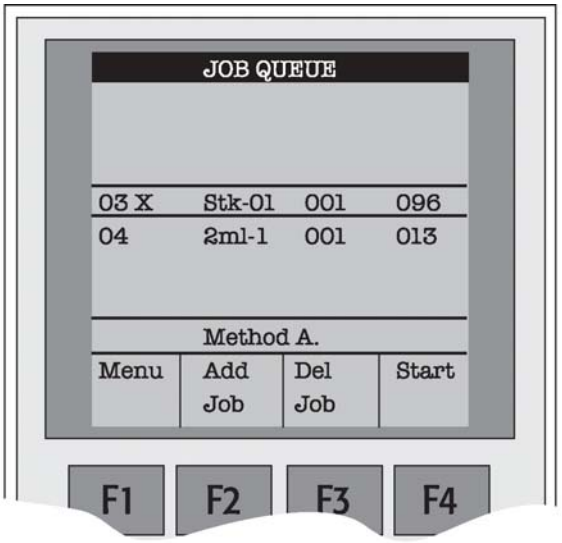


Figure 4 Example Job Queue Screen

2 Load a sample Tray onto an available location in a Tray Holder or Stack. Note the corresponding Tray name.

3 Add a new Job for the Tray. Press the **Add Job** key to bring up the default Job.

4 For **TRAY**, select the Tray name (e.g. Stk1-01) that corresponds to the location of the Tray that was just loaded.

5 Enter the *First* and *Last* sample number for this Job.

6 Select and enter the sample processing *Method* for this Job.

7 Press the **Home** function key (**F4**) to return to the **JOB QUEUE** screen.

8 To add additional samples to be processed, repeat steps 2 - 7.

9 If necessary, replace and/or clean the syringe (liquid versions only). Press the **Menu** key to see the available options for changing (**F1** - **Change Syringe**) and cleaning (**F2** - **Clean Syringe**). To completely remove air bubbles, the syringe should be primed manually.

10 If only one Job is to be processed, select the Job with the scroll knob. Press **Start** key. In the dialog box "**Select Job(s) to Process**" select one of the following options:

- **All** (Entire Job Queue starting from the top)
- **Selected** (Job selected with the cursor bar)
- **Resume** (Continue with the next Job after the one aborted)

Aborting a Job Queue

1. Press **STOP**
2. Select one of the available options (**Continue**, **Sample**, **Job**, or **Job Queue**).
   - Select **Continue** to resume processing with the current sample.
   - Select **Sample** if there is a problem with only the current sample. Processing will resume with the next sample.
   - Select **Job** to abort processing all samples in the current Job. Processing will resume with the next Job. The aborted Job is marked with an **X**.
   - Select **Job Queue** to abort processing all Jobs. The **JOB QUEUE** screen will be displayed. The aborted Job is marked with an **X**.

Restarting an Aborted Job Queue

1. Press the **START** key.
2. Select the **Resume** option. The Job after the last one marked as aborted will be started.

Utility Functions

Utility functions, selectable from the **Menu** screen, provide quick access to checking operations and parameters that may need to be changed. These functions are available for the actual Syringe, Trays, Injectors, and the Wash Station. They allow access to key functions without having to set up and execute a Method and Job.

NOTE If an item is used in the sample processing cycle, the appropriate **Utility** value will be overwritten by the **Method** value.

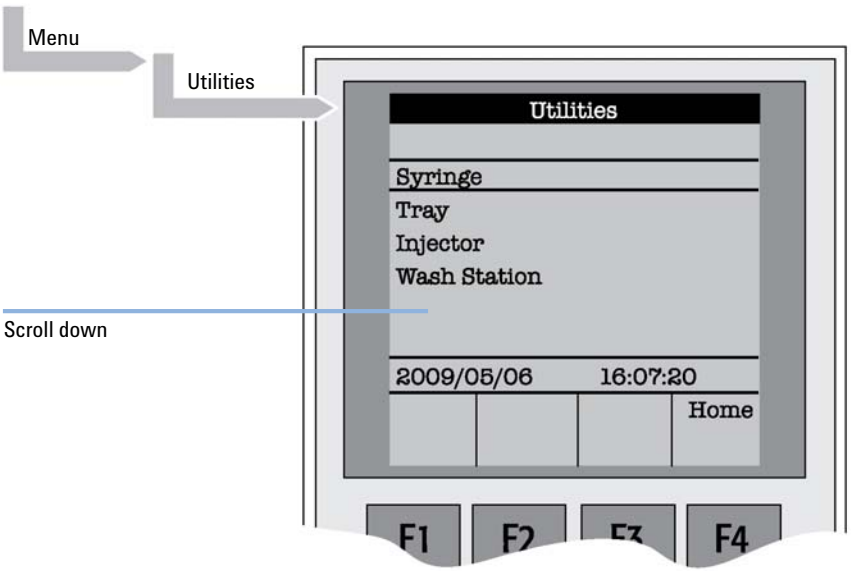


Figure 5 Selecting Utilities Functions

Syringe

The following functions are available by pressing a Function Key:

Table 2 Syringe - Function Key

Function Key	Description
F1Chang Syr	The syringe is moved to a position in which the syringe assembly can be completely lowered to facilitate removal of the syringe adapter. The syringe can then be removed from the adapter and replaced. A prompt will be displayed to specify the new syringe. The syringe must be installed before pressing **Enter**.
F2Clean Syr	This Function is used to clean or prime the syringe prior to use. After selecting **F2** either **Wash1** or **Wash2** can be selected.
F3Set Pos	**Set Pos** is used to define the Chang Syr position
F4HOME	The Injection Unit moves to its **HOME** position and the **Job Queue** Menu is displayed.

The following Syringe items may be changed by selecting the particular item:

Table 3 Syringe Items

Item	Description
Actual ID	Indicates the identification number (ID) of the currently inserted syringe. If the syringe detection system is set to manual, the message **Syringe: No syringe** is displayed.
Fill Volume	This parameter serves to control the filling of the syringe. It can occur that air bubbles remain below the plunger after the first pull up. If the plunger is moved up and down several times (see Fill Strokes), these air bubbles are worked out. With this operation the syringe can be completely filled even when using very small sample volumes.
Fill Strokes	Number of fill strokes. All fill strokes, except the last one, use the selected fill volume. If the selected sample volume is higher than the fill volume, the sample volume is used for all fill strokes. If zero is selected the plunger is pulled up only once using the sample volume value.
Pullup Del	By using this item, a delay time can be selected between sample pullup and ejection while filling the syringe. When the plunger reaches the zero position during the fill strokes, the system waits half the **Pullup Del** time. This allows for an air bubble to float away from the needle tip. This feature is especially useful for removing any air bubble in the syringe and handling viscous fluids.
Fill Speed	Speed of plunger movement used in all syringe filling operations.
Eject Speed	Speed of plunger movement used in all syringe eject operations except sample injection.
Inject Speed	Speed of plunger movement for sample injection. Typically used for **Fill Strokes**.
Plunger Chnge Pos	Plunger position during **Change Syringe** operation. The syringe plunger is moved to a position where the syringe can be removed and replaced. The value may be changed for different types of syringes.

Tray

After selecting the particular Tray to be accessed, the following functions are available:

Table 4 Tray - Function Key

Function Key	Description
F2Block NdlG	**F2** activates Needle Guide blocking. The option after activation is "**Rel NdlG**", releasing Needle Guide Blocking. It may be used to test the functionality of the solenoid that blocks the needle guide.
F3Movto nnn	This function serves as a quick check to determine if the X,Y,Z-coordinates are defined correctly for the selected Tray. To use this utility the selected Tray, including the sample vials, must be present. After pressing "**Movto 001**" the Injection Unit moves to sample position no.1. This procedure can be repeated for the last sample position in the first row and the last sample position.
F4HOME	The Injection Unit moves to its **HOME** position and the **Job Queue** Menu is displayed.

The following Tray items may be changed by selecting the particular item:

Table 5 Tray - Items

Item	Description
Needle Penetr	Needle penetration depth into the sample vial. The needle penetration depth for the selected Tray can be changed by entering the desired value.
Tray Type	The Tray Type which is selected for the Tray is shown. If the Tray enables the use of different Tray Types it can be changed at this position.
Tray Offset X	If necessary, a correction to the ideal X-position of "Position 1" can be made by using "**Tray Offset X**".
Tray Offset Y	If necessary, a correction to the ideal Y-position of "Position 1" can be made by using "**Tray Offset Y**".
Tray Offset Z	If necessary, a correction to the ideal Z-position of "Position 1" can be made by using "**Tray Offset Z**".
dxRow[1]	Correction of any inclination of a Tray (plate) in the X-axis of a row (see Figure 37 on page 90 for details).
dyRow[1]	Correction of any inclination of a Tray (plate) in the Y-axis of a row (see Figure 37 on page 90 for details).
dzRow[1]	Correction of any inclination of a Tray (plate) in the Z-axis of a row (see Figure 37 on page 90 for details).
dxCol[1]	Correction of any inclination of a Tray (plate) in the X-axis of a column (see Figure 37 on page 90 for details).
dyCol[1]	Correction of any inclination of a Tray (plate) in the Y-axis of a column (see Figure 37 on page 90 for details).
dzCol[1]	Correction of any inclination of a Tray (plate) in the Z-axis of a column (see Figure 37 on page 90 for details).

[1] Available beginning with PAL Firmware Level 4.1.X.

NOTE A "staggered pattern" is selectable in firmware class "**Tray Type**". For details see "Tray Type" on page 138.

Injector

After selecting the particular Injector to be accessed, the following functions are available:

Table 6 Injector - Function Key

Function Key	Description
F3Movto Inj	The Injection Unit moves to the selected injector position. With this function, e.g. the injectors **LC Vlv1**, **Waste** and **Waste2** can be accessed. By selecting the parameter "**Needle Penetr**" on the same screen, the Injector Needle Penetration value can be checked or changed.
F4HOME	The Injection Unit moves to its **HOME** position and the **Job Queue** Menu is displayed.

The following Injector item can be changed by selecting the particular item:

Table 7 Injector - Parameters

Item	Description
Needle Penetr	By selecting the parameter "**Needle Penetr**" the Injector Needle Penetration value can be checked and/or changed. To ensure reproducible sample injections and minimize carry-over it is critical that the needle penetration depth be accurately set.

Wash Station

After selecting the specific Wash Station, the following Functions are available:

Table 8 Wash Station - Function Key

Function Key	Description
F3Movto Wash	The injection unit moves to the selected Wash Station port. By selecting the Parameter "**Needle Penetr**" on the same screen the Wash Station Needle Penetration value can be checked or changed.
F4HOME	The Injection Unit moves to its **HOME** position and the **Job Queue** Menu is displayed.

The following Wash Station Parameter may be changed by selecting the particular item:

Table 9 Wash Station - Items

Item	Description
Needle Penetr	By selecting the Parameter "**Needle Penetr**" the Wash Station Needle Penetration value can be checked and/or changed.
Rinse Time	If **Rinse Time** is activated (value > 0), the solenoid of a Fast or Active Wash Station opens for the specified time after the syringe needle has been removed from the wash port (after completion of the syringe wash cycle). The solvent flows into the wash port without the restriction of the needle; be aware of higher solvent consumption.

Vial

After selecting the specific Vial type, the following functions are available:

Table 10 Vial - Function Key

Function Key	Description
F3Movto Vial	The injection unit moves to the selected vial type. By selecting the parameter "**Needle Penetr**" on the same screen, the Wash Station Needle Penetration value can be checked or changed.
F4HOME	The Injection Unit moves to its **HOME** position and the **Job Queue** Menu is displayed.

Table 11 Vial - Items

Item	Description
Needle Penetr	By selecting the parameter "**Needle Penetr**" the Wash Station needle penetration value can be checked and/or changed.

Dilutors

After selecting the specific Dilutor, the following functions are available:

Table 12 Dilutors - Function Key

Function Key	Description
F1Prime	The Dilutor syringe is primed with solvent after activating the **F1** function.
F2Chang DSyr	Activating **F2** moves the plunger of the Dilutor Syringe to standby position to allow easy access to change the syringe.
F4HOME	The Injection Unit moves to its **HOME** position and the **Job Queue** Menu is displayed.

The following Dilutor items can be changed by selecting the particular item:

Table 13 Dilutor - Items

Item	Description
Syringe	Indicates the Dilutor side-port syringe inserted in the Z-axis. This function allows selecting another syringe size.
Syr Dilut Pos	Activating this function moves the plunger of the side port syringe up by the specified distance. This allows adjustment of the plunger tip of the side port syringe exactly above the lower side port (solvent inlet). This fine tuning is necessary after changing a side port syringe to allow unrestricted solvent flow.
Dilutor Syr	Indicates Dilutor Syringe is installed. If the syringe size is changed, this item must be adapted accordingly. The syringe-specific dimensions are coordinated by this function.
Prime Volume	A volume to prime the Dilutor Syringe can be specified. The allowed range can be fourfold higher than the actual syringe volume. This allows filling and emptying a dilutor syringe more often than just once.
Pullup Delay	Using this item permits selecting a delay time between solvent filling and ejection while filling (or priming) the syringe.
Fill Speed	Speed of plunger movement of Dilutor Syringe used in all syringe filling operations.
Eject Speed	Speed of Dilutor Syringe plunger movement used in all dilutor syringe eject operations.
Eject Delay	Using this item permits selecting a delay time between solvent ejection and filling while filling (or priming) the syringe.

Tools

NOTE The Object class **Tools** is available with Firmware version 3.0.X , 4.1.X. or higher.

After selecting the specific tool, the following functions become available:

Table 14 Tools - Function Key

Function Key	Description
F1Chk Offs	The item "*Check Offset*" can be used to verify the offset from the syringe needle tip to the tool tip. This item is mainly used for the **MALDI** tool; it is not active for the **MHE** tool.
F4HOME	The injection unit moves to its **HOME** position and the Job Queue Menu is displayed.

The following tool items may be changed by selecting the particular item:

Table 15 Tools - Items

Item	Description
Teach Point	The reference point at which to teach the Object (**Tool**; **MHETool**) is selectable. In the case of the **MHETool**, no extra position is necessary. The Parking Station is the fix point. Select "**None.**"
ToolOffset X	If necessary, a correction to the ideal X-position of the **MHETool** can be made by using "**ToolOffsetXX**".
ToolOffset Y	If necessary, a correction to the ideal Y-position of the **MHETool** can be made by using "**ToolOffsetY**"
ToolOffset Z	If necessary, a correction to the ideal Z-position of the **MHETool** can be made by using "**ToolOffsetZ**" A "**ToolOffsetZ**" of -48 mm is necessary to position the **MHETool** on the sample vial.

Logfile

The logfile cannot be read-out directly on the terminal display.

However, **F3** does allow a print out. A serial printer has to be connected to port SER2. If a serial printer is not available use a serial/parallel converter.

Info Functions

Info functions, selectable from the Menu screen, provide quick access to information to be viewed. These info functions are available for the **Hardware**, **Software**, **Maintenance**, **Free Objects**, and **Free Items**. All items with the exception of "**Maintenance**" are read only.

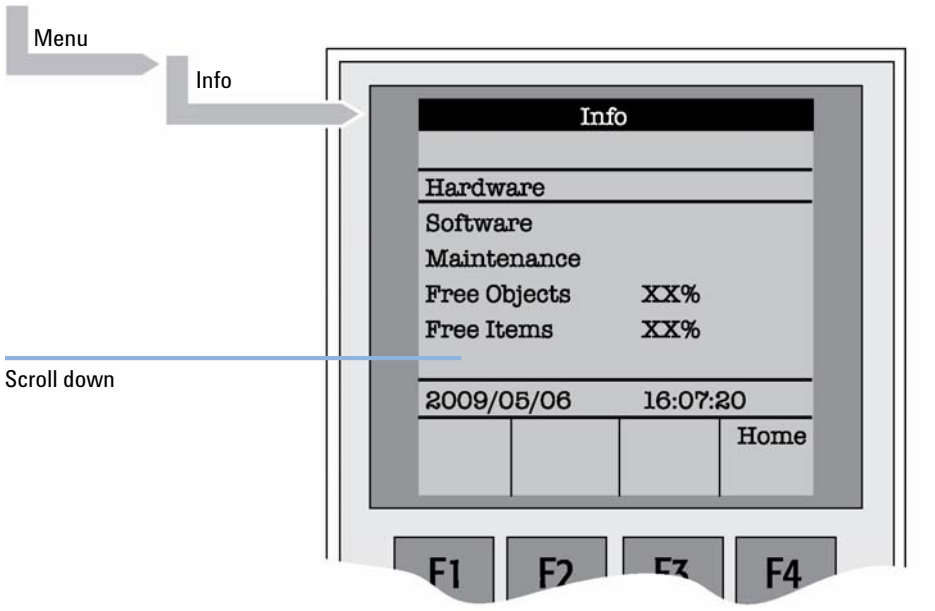


Figure 6 Selecting Info Functions

Hardware

Table 16 Hardware

Item	Description
CPU SNo	The serial number (SNo.) of the PCB “APR CPU” is displayed.
CPU ID	Version number of the PCB “APR CPU”.
MOTIO ID	Version number of the PCB “APR CPU”.

Software

Table 17 Software

Item	Description
Firmware	Firmware version of System
Head Firmware	Firmware version of the Injection Unit
Terminal FW	Firmware version of the Terminal
Altera Firmware	Firmware version of the Altera component

Maintenance

Table 18 Maintenance

Item	Description
PlgStrokeCnt	This is a counter for syringe plunger movements. The actual number of strokes is displayed. If the counter reaches the set limit (**PlgStrkeLim**) a warning is displayed at the next start of a job (run). The system continues but signals the user to verify syringe conditions. The counter can be set back to zero to restart. There is only one counter for a syringe. If syringe types are changed the system continues to count as if it were the same type.
PlgStrokeLim	An upper limit for the syringe plunger strokes can be set.
Inject Count	This counter monitors the number of injections. The number of injection valve switches is a helpful tool for the user to decide on a replacement of parts of the injector system. The actual number of injections (valve switches) is displayed. If the counter reaches the set limit (**Inject Limit**), a warning is displayed at the next start of a job (run) (**Inject Limit**). The same counter is used for injector penetrations with GC technique.
Inject Limit	An upper limit for the number of injections can be set.

NOTE Counters for the plunger movement and injector penetrations are available with LC Injector HTC/HTS Firmware version 2.5.X or higher.

Free Objects / Free Items

In addition to the core software, the Firmware contains data for the **Firmware Objects**. There are different classes of Objects, such as Syringes, Trays, Tray Holders, etc. Each class of Objects contains **Items**. The items contain the actual data such as X-, Y-, Z-positions.

The data are stored in a flash memory backed up by a battery. To optimize RAM and Flash memory use, a certain section of memory has been reserved by the software for each of the Objects and Object Items.

The percentage shown in the **Info** section provides an indication as to how much of the reserved software space is still available.

Setup Functions

The **Setup** functions, selectable from the Menu screen, allow access to various functions for the sampler system. The **Sound**, **Time** and **Objects** are basic functions used at installation or if changes have been made over time.

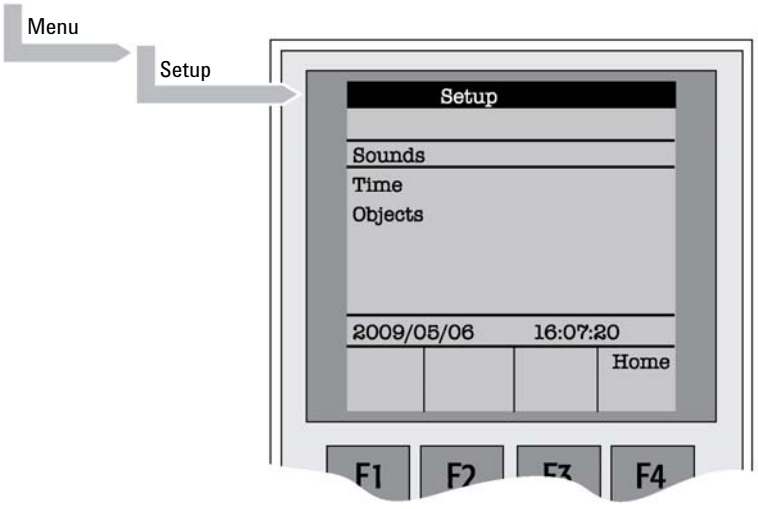


Figure 7 Selecting Setup Functions

Sounds

Table 19 Sounds

Item	Description
Message Box	A specific dual beep tone signals that a pop-up window (Message Box) for user intervention appears on the screen. This beep signal can be turned on or off.
Warn Move	A beep sound is heard at the start of the module movement. It is advisable to keep this function turned on for safety reasons.
End Cycle	A beep sound is heard at the end of a cycle. Select as desired.
End Job	A beep sound is heard at the end of a cycle. Select as desired.

Time

Table 20 Time - Item

Item	Description
Year	The year can be set for the module internal clock
Month	The month can be set for the module internal clock.
Day of Month	The day can be set for the module internal clock.
Hours	The hours can be set for the module internal clock.
Minutes	The minutes can be set for the module internal clock.
Seconds	The seconds can be set for the module internal clock.

After setting or resetting the date and time, use **F1**

"**Set Time**" to store.

Objects

The various module Firmware Object classes can be selected and the functions most used by the user are directly accessible.

For detailed listing see “LC Injector HTC/HTS-xt System Flow Chart based on PAL Firmware version 4.1.X (HTC)” on page 246.

3
Description and Installation

This chapter gives an overview and information about the installation of your Agilent 1290 Infinity LC Injectors HTC/HTS.

General System Overview

NOTE Agilent reserves the right to make improvements and/or changes to the product specifications without notice.

The LC Injector HTC/HTS includes the following hardware:

1 X-, Y-axes assembly;

1 Injection Unit, Z-axis;

1 Dynamic Load an Wash Option;

1 1200 bar Injection Valve

1 Keypad terminal;

1 Safety Guard;

1 Standard standalone supports, 215 mm.

Optional TraySets (Tray Holder and Tray) and/or Stacks of various types and in different variations are required and can be added.

Specifications

Table 21 Specifications

Type	Specification
System	XYZ robot with syringe-only concept, no tubing in sample path
Sample capacity	**HTC Sample capacity** Up to 24 shallow well-plates (96 or 384) Up to 12 deep well-plates (96 or 384), or 648 2 mL vials Up to 12 shallow well-plates (96 or 384), thermostatted Up to 6 deep well-plates (96 or 384), or 324 2 mL vials, thermostatted **HTS Sample capacity** Up to 24 shallow well-plates (96 or 384) Up to 18 deep well-plates (96 or 384), or 972 2 mL vials Up to 12 deep well-plates (96 or 384), or 648 2 mL vials, thermostatted
Syringe Size	100 µL
Loop sizes for DLW and maximum pressure rating	2 µL (standard) 1200 bar max., 5 µL, 10 µL, 20 µL1000 bar max., 50 µL and 100 µL600 bar max.
Minimum Sample Volume	1 µL from a 3 µL sample in 1 mL tapered micro vial in liquid injection mode
Injection volumes	Up to 100 µL with standard setup, up to 5 mL with additional hardware
Injection precision	<0.5 % with 100 µL syringe and 2 µL loop, overfill 7 µL
LC Injector	1 electrically actuated fast switching 6-port valve from Rheodyne (1200 bar)
Needle, syringe valve cleaning	Dynamic load and wash (DLW) principle: two different solvents Other wash options are available
Carryover	Typically < 0.004 % with DLW Method: Chlorohexidine, 0.6 mg/L dissolved in H_2O : ACN = 90 :10 , 0.1 % TFA Blank Solution: H_2O : ACN = 90 :10 , 0.1 % TFA
Local control	Handheld control panel with 4 function keys, graphical LCD display
Electronic control	• 2 RS232C ports • 3 TTL inputs • 1 opto-coupler input • 2 relay outputs
Thermal control	4 – 40 °C (settable)

Table 22 Specifications DLW-2 Option

Type	Specifications
DLW Pumps	Two pcs. solenoid diaphragm pump, self-priming - Flow rate range: 100 mL/min at atmospheric pressure (Pump itself, no connections to HPLC system) - Wetted parts Pumps: Ryton PPS and Kalrez (FFPM)
Transfer Tubing Kit	Two pcs. 1/8" PFA tubes including connection fittings
Solvent Reservoir	1000 mL borosilicate glass including Glass Filter, 40 µm pore size
DLW Wash Station	Wash Station with two solvent ports and one Waste port. Two additional horizontal Waste inlet ports in front to be combined with Valve waste port. • Wetted parts: ◦ Wash Station block: PVDF ◦ Inserts: sst 1.4404 ◦ Connections at bottom of inserts: PEEK ◦ Waste Tube: Polyethylene (PE)
DLW Syringe Assembly	• Syringe: Gastight Syringe 100 µL with Valflon tip ◦ Wetted parts: Glass, PTFE and Valflon • Syringe Needle: ◦ Gauge 22 (OD 0.72 mm/ID 0.41 mm) Length: 0.51 mm, PTFE Seal • DLW Actuator/ 2/2-way flipper solenoid valve ◦ Solenoid: ◦ Wetted parts: ◦ Body: PEEK ◦ Seal material: FFKM (Simriz) • DLW Manifold Solvent selector module and Syringe adapter assembly ◦ Wetted parts: ◦ Block: sst 1.4435 ◦ Perfluor (O-ring) • Holding Loop: Stainless steel Tubing, OD 0.72 mm, ID 0.41 mm(Gauge 22) Length 85.5 cm; Loop Volume: 118 µL Internal surface acid passivated.

Performance Specifications

The performance specifications are available as part of a specific, separate document.

Electrical Specifications

Table 23 Electrical Specifications

Parameter	Requirement
Protection Class[1]	Class I
Over Voltage Category[2]	Category II
Pollution Degree[3]	2
Moisture Protection[4]	Normal (IPXO)
Voltage	36 VDC
Current	3.2 A
Fuse	T6.3 A/250 V
System Power Supply	
Input line voltage	Grounded AC, 100 to 240 V
Input line frequency	50 /60 Hz
Input power	4 A
Output voltage	36 VDC
Output current	4.16 A

1 Protection class describes the insulating scheme used in the instrument to protect the user from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn this grounding system is connected to the third pin (ground pin) on the electrical power plug.

2 Over Voltage category II pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.

3 This is a measure of pollution on electrical circuits that may produce a reduction of the dielectric strength or surface resistivity. Degree 2 refers to normally only non-conductive pollution. Occasionally, however, a temporary conductivity caused by condensation must be expected.

4 Normal (IPXO) – IPXO means that there is NO Ingress Protection against any type of dripping or sprayed water. The X is a place holder to identify protection against dust if applicable.

Physical Specifications

Table 24 Physical Specifications

Parameter	Requirements
LC Injector HTC	
Height	648 mm (25.5 in)
Depth	385 mm (15.2 in)
Width	545 mm (21.5 in)
Weight	8 kg (18 lbs.) without accessories
LC Injector HTS	
Height	648 mm (25.5 in)
Depth	385 mm (15.2 in)
Width	828 mm (32.6 in)
Weight	10 kg (22 lbs.) without accessories

Operating and Environmental Requirements

Table 25 Operating and Environmental Requirements

Parameter	Requirements
Operating Temperature Range	4 to 40 °C (39 to 104 °F)
Maximum Relative Humidity	75 %, non-condensing
Bench Space	At least 24 cm (10 in.) at the rear. Access to power switch(es) and power cord(s). Clean, level and smooth surface. Solid bench plate.
Vibration	Negligible
Static electricity	Negligible

Sound Pressure Level

Table 26 Sound Pressure Level

Parameter	Requirements
Sound Pressure Level	Measured value: 62 dBA[1] (PAL System used for measurement) One meter from the equipment in the direction of maximum sound pressure level. According to UL 610107A-1, 1st edition, clause 12.5. Limit < 85 dBA

[1] dBA = "A weighted" sound pressure level

Software Requirements

The various PAL Software programs, such as PAL Loader or PAL Object Manager, are operated with Microsoft Windows Operating systems, such as Windows XP and Vista.

The Agilent 1290 Infinity LC System can be controlled using PAL control software, or by Agilent LC/LCMS ChemStation, Masshunter for LCMS and EZChrom.

Regulatory Compliance Requirements

As of the date of publication, this product is compliant with current RoHS and WEEE regulations.

- Directive 2002/95EC, RoHS
- Directive 2002/96/EC, WEEE

NOTE Agilent Technologies reserves the right to make improvements and/or changes to the product specifications without notice.

Installation

Unpacking the Components

An autosampler system is shipped in several boxes. One box contains the X-,Y-axes assembly, the Injection Unit, the Keypad Terminal, standalone Supports, connecting cables, Power Supply, 1200 bar Injection Valve including valve drive, Safety Guard and miscellaneous parts. The Dynamic Load and Wash accessories and all optional parts, such as TraySet, Stack Cooler, second valve drive assembly, etc., are shipped in separate boxes.

1. Open the box and first remove the accessory boxes and the Injection Unit before attempting to remove the X-, Y-axes assembly.
2. Carefully lift the X-,Y-axes assembly and remove it from the box. Hold the Y-axis in place while the assembly is being removed from the box. Set the X-,Y-axes assembly on a bench.
3. Unpack the remaining small boxes and any other accessories.
4. When placing the LC Injector HTC/HTS onto a stable surface, make sure that no objects interfere with either the Y-axis or the Injection Unit throughout the entire range of potential movement.

Assembling the LC Injector HTC/HTS

Before beginning the assembly process, determine approximately where the LC injection valve and keypad terminal will be located. The terminal may be mounted on either side of the X-axis.

1 If a Stack (a Tray Holder with multiple drawers) was shipped with the LC Injector HTC/HTS, loosen the Torx screws on the two mounting clamps located on top of the stack.

2 Move the X/Y-Carriage to the center of the X-axis and temporarily place the X-axis assembly on top of the Stack with the mounting clamp teeth fitting into the grooves on the bottom of the X-axis.

NOTE For Systems without a Stack, support the X/Y-Carriage in a suitable manner (a sturdy cardboard box can be used) before attempting to install the legs.

3 Install the legs near the ends of the X-axis. Loosen the Torx screws on the mounting clamps and then fit one leg into the grooves in the X-axis. Be sure that the clamps fit completely into the grooves. Tighten the Torx screws until the legs are firmly in place.

4 Double check whether the leg claws are correctly attached to the X-axis (see Figure 8 on page 55).

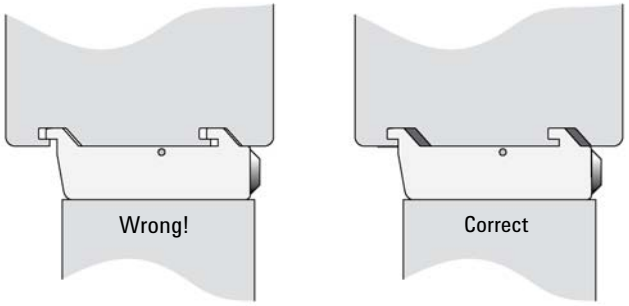


Figure 8 Attachment of Mounting Claws

Installing the Injection Unit

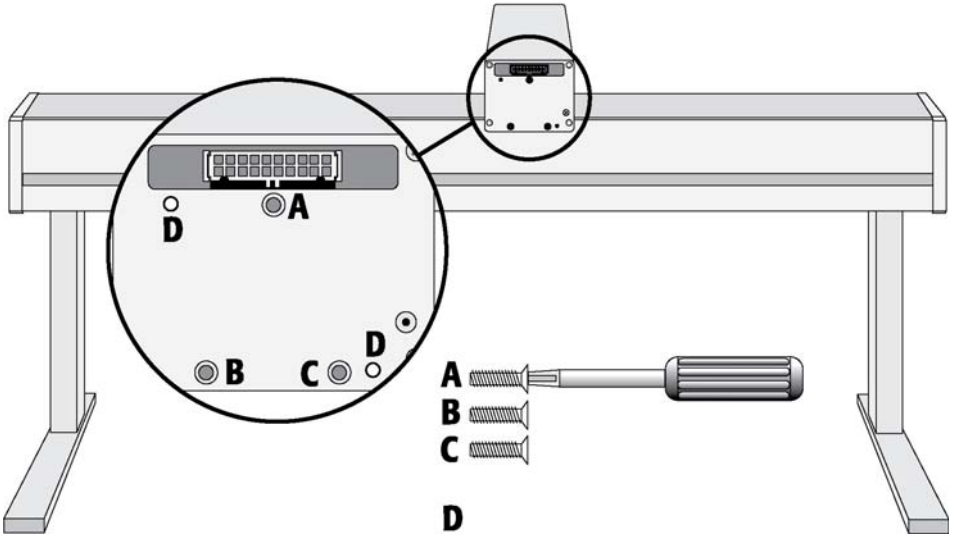


Figure 9 Attaching the Injection Unit (shown freestanding for clarity)

NOTE Installation of the Injection Unit should be done carefully. When installing it for the first time, have someone hold it in place while the mounting screws are inserted.

(See Figure 11 on page 58).

1 Remove the three Torx mounting screws *A*, *B*, and *C*, used to fix the Injection Unit to the Y-axis

2 Connect the ribbon cable (see Figure 10 on page 57).

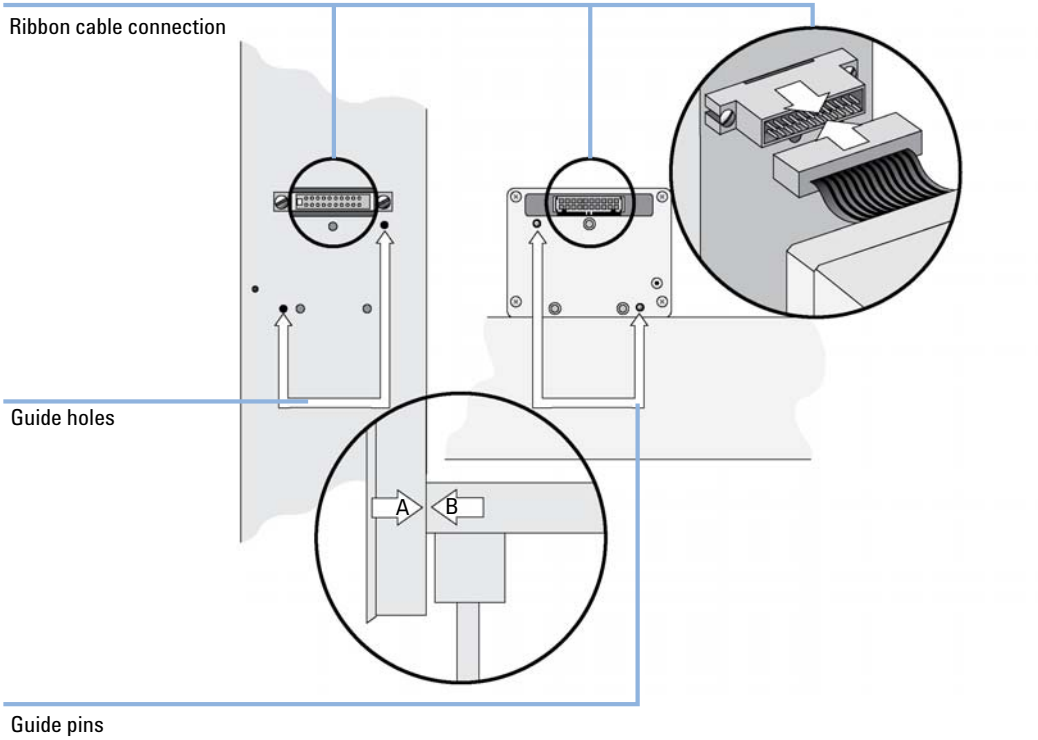


Figure 10 Connecting the Injection Unit Ribbon Cable

3 Hold the Injection Unit in place against the Y-axis. Make sure the two locating pins on the Y-axis fit into the two guide pin holes on the Injection Unit.

4 Place one of the screws onto the end of the supplied Torx driver. Slide the clear plastic cover on the Injection Unit all the way to the top. Locate the three large holes in the black anodized frame attached to the Z-axis inside the Injection Unit. Slide the frame upwards until the top hole is centered on the top threaded hole at the end of the Y-axis. Insert and securely tighten the Torx screw *A* (see Figure 11 on page 58).

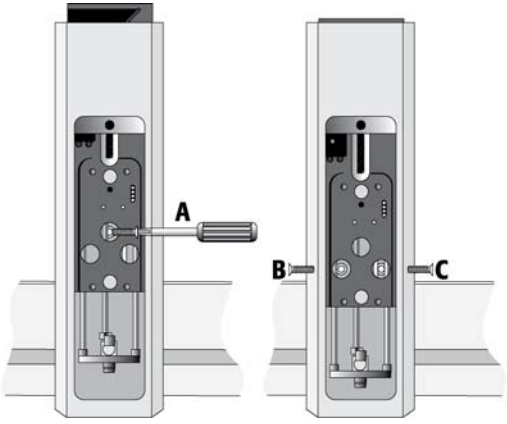


Figure 11 Inserting the Injection Unit Mounting Torx Screws

5 Install the two remaining Torx screws *B*, *C* in the left and right mounting holes, respectively. It may be necessary to move the elastic cord slightly to the left to insert the Torx screw *C* into the right-hand hole.

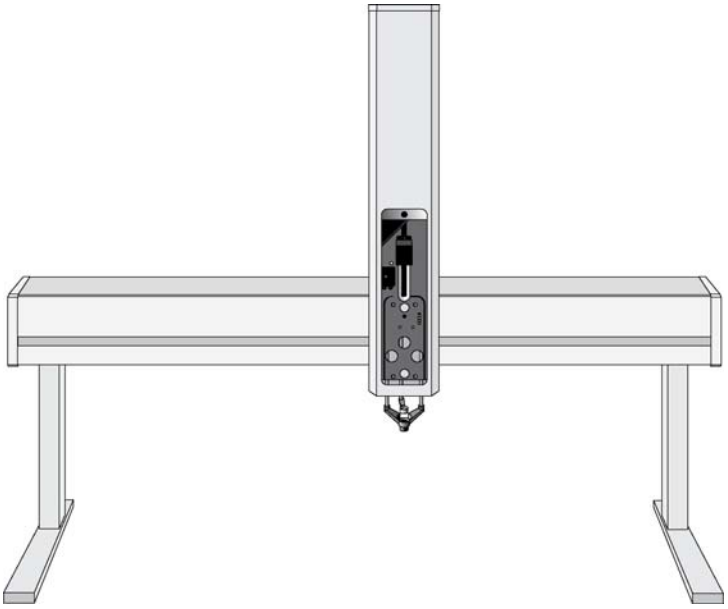


Figure 12 LC Injector HTC/HTS with Injection Unit and Standalone Supports

Installing the Keypad Terminal and Safety Shield

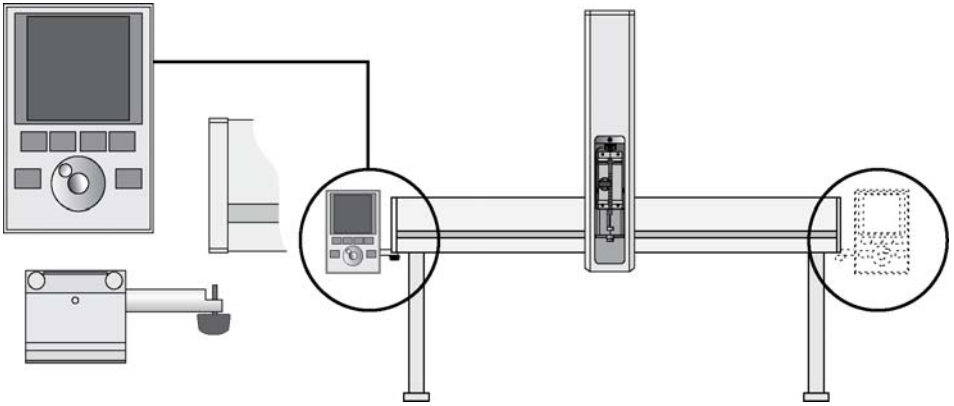


Figure 13 Installing Keypad Terminal

1 Install the safety shield on the left and right sides to the outside of the X-axis. Use the provided, longer thumbscrew on the side where you plan to install the keypad.

2 Install the Keypad mounting bracket on either the right or left side of the X-axis.

3 Connect one end of the white coiled cable (Part No. SS8J-700) to the Keypad and the other end to the TERMINAL (SER3) interface jack on the rear side of the X-axis. For details see Figure 33 on page 86 or Figure 34 on page 87.

4 Place the Keypad terminal onto its mounting bracket.

NOTE Do not interchange the Terminal with LAN cable connectors.

Installing the Power Supply

1 Locate the power supply, the DC power cable, and the AC power cable.

2 Set the power supply switch to the *OFF* position.

3 Connect one end of the DC power cable to the power supply and the other end to the *POWER* connector at the rear side of the X-axis.

4 Connect the female end of the AC power cable to the power supply. Then connect the male end to an AC power outlet.

Installing the LC Injection Valve

1 Locate the blue, valve drive. It will have one clamp that is identical to other object clamps. Loosen the clamp as above (see Figure 14 on page 61).

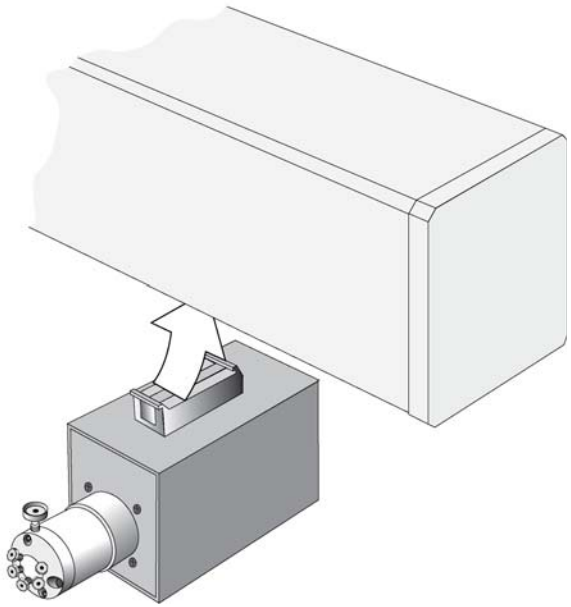


Figure 14 Installing the Injection Valve Drive

2 Attach the valve drive to the X-axis and tighten the mounting screw.

NOTE To avoid delay volume between sample injection point and detection, the injection valve should be located near the detection device.

3 Connect the control cable from the valve drive to AUX1.

NOTE The installation of another type or multiple Valve Drive(-s) is described in "Valve Drives and Valves – General Remarks" on page 105.

4 The injection valve and rotor are pre-installed on the valve drive.

Installation of the DLW Option

General System Overview

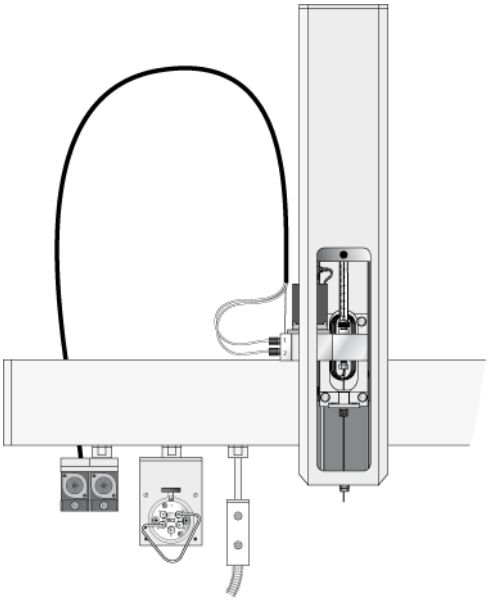


Figure 15 DLW-2 Option Components

DLW: Dynamic Load and Wash

The DLW Option represents a new concept in wash stations, combining an injection cycle with wash steps. The combination of both injection and wash steps – usually separate procedures – minimizes cycle time and carry-over.

The two characteristic features of the DLW Option are:

- The sample solution never comes in contact with the syringe itself; it is held ‘sandwiched’ in a so-called Holding Loop.
- Wash solvents are pumped from the rear to the front of the DLW system, to intensely purge all critical surfaces which have come in contact with the sample.

The DLW option consists of two self-priming micro pumps (mounted on a dedicated bracket), the wetted parts are composed of Ryton PPS and Kalrez (FFPM). The ‘IN’ ports are connected to the wash solvent bottles and the ‘OUT’ ports are connected to the DLW manifold, which is part of the dedicated DLW Syringe Holder assembly. A ‘Holding Loop’ separates the syringe and the DLW actuator to avoid the sample coming in contact with these parts.

TThe syringe and Holding Loop are preloaded with Wash Solvent #1 at the start. The sample is picked up and remains separated from Wash Solvent #1 by an air gap. After loading the loop and injection, Wash Solvent #1 is pushed into the system, followed directly by Wash Solvent #2 to flush the critical valve paths.

The DLW Syringe Assembly is then moved to the wash station for further cleaning steps and to prepare the Syringe and Holding Loop for the next cycle.

Further details are given in “DLW Cycle Step-by-Step” on page 211.

Development of the PAL DLW Option

The DLW Option has undergone several development steps. The current version, DLW-2 Option, contains several improvements over the earlier versions:

- Holding Loop

  The DLW-2 Holding Loop consists of one single piece from the needle to the loop. This eliminates several tube connections, the replacement needle and the Flow diverter. The loop is made of high quality stainless steel and the inner surface is passivated with acid.

- DLW Syringe Holder Back-plate

  The DLW-2 Syringe Holder back-plate has changed in size and form to allow a smoother replacement of the holding loop. The Actuator is supported from the backside to provide higher mechanical stability.

- DLW Pump Assembly

  The DLW-2 Pump assembly is newly equipped with a housing to protect the electrical parts from solvent splashes.

NOTE This Manual covers the DLW-2 Option. The technique as such has been established with previous versions and is called the 'DLW technique'. Most of the parts are used for the 'DLW Option' as well as for the 'DLW-2 Option'. DLW-2 specific parts or their usage are emphasized. Otherwise, the general term 'DLW' is used which applies to the earlier versions and the current version 'DLW-2'.

Hardware Requirements

The DLW option can be used with Agilent 1290 Infinity LC injectors in combination with the Rheodyne ultra high pressure valves.

Software Requirements

The DLW option can only be operated with Firmware 1.2 or higher. It can be controlled using PAL Control software, the Cycle Composer or any CDS (chromatography data system) software that controls the PAL System including those using the Cycle Editor for PAL ICC interpretation.

Unpacking the Components

The DLW-2 option is shipped in one box. Check for the following items:

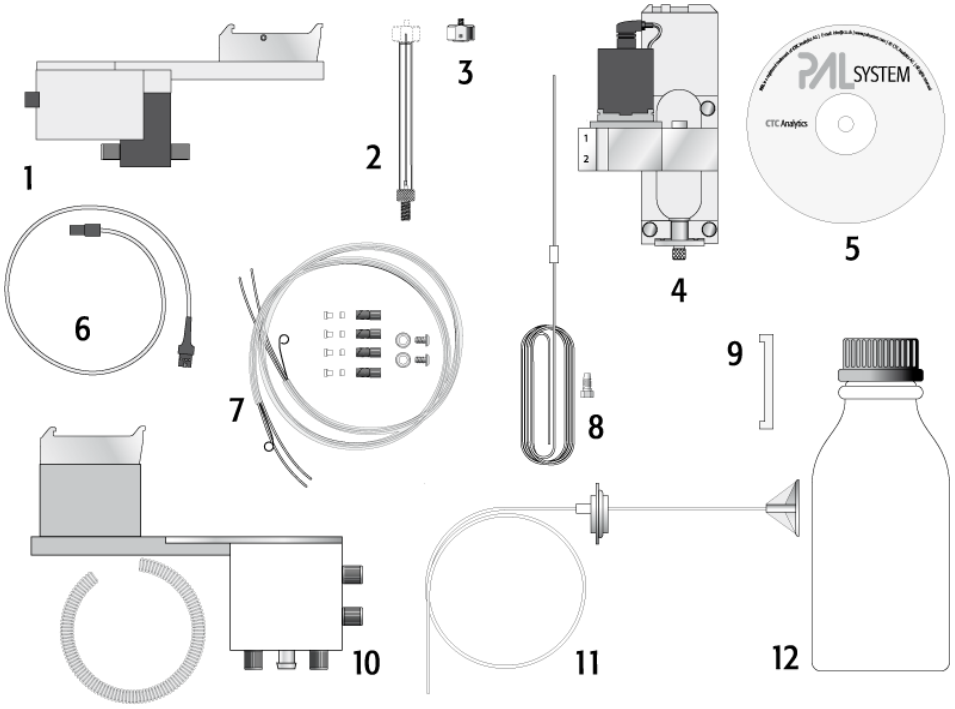


Figure 16 DLW-2 Option Components

Item	p/n	Description
1	G4277-60510	DLW Pump Module (re-order for one solvent channel)
2	5190-3259	DLW Syringe
3	G4277-60509	DLW Plunger Holder
4	G4277-60501	DLW Syringe Holder Assembly
5		CD ROM
6	G4277-60513	Wash Station cable to System
7	G4277-60512	DLW Tubing Kit
8	G4277-60602	DLW Holding Loop (installed in the DLW Syringe Assembly)
9	G4277-60515	Needle Guide Length Tool
10	G4277-60514	DLW Wash Station incl. Waste Tube
11	G6500-88035	Solvent Bottle Transfer Line including Glass Filter Solvent Inlet, 40 µm pore size
12	(2x)	1 L Solvent reservoir bottles

Please refer to Figure 15 on page 62 for an overview of how the various DLW components are mounted on the system.

Mounting the Pump Holder

The pump holder should always be mounted on the far left of the X-axis. The solvent lines are connected to the pump holder. Moving across the pump holder with the Z-axis could kink the stabilizing wire. To install the holder, it is recommended to keep the DLW syringe module out of Z-axis.

1 Locate the pump holder and the two supplied Torx screws.

2 Attach the holder to the X-axis as shown in Figure 17 on page 68.

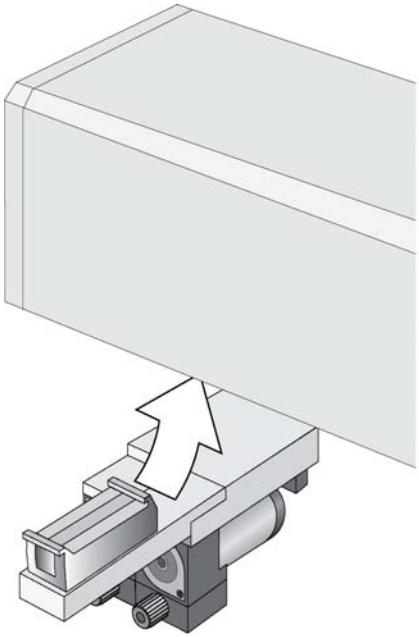


Figure 17 Attaching Pump Holder Bracket to X-Axis

3 The Solvent line assembly is not preinstalled at the factory. The correct mounting is shown in Figure 18 on page 69. Select the end of the guide wire with the longer tubing ends and connect the guide wire with the screw M4 x 8 and the corresponding serrated lock washer to the back side of the module. Note the guide groove, which orients the wire.

NOTE Always install the DLW Tubing (from the kit) to the pump module first. The other end, connected to the DLW Syringe Assembly, should be connected while the assembly is not yet installed onto the injection unit.

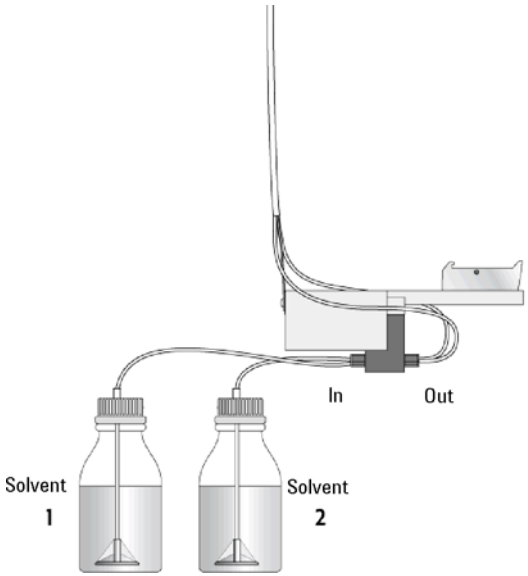


Figure 18 Installing the Solvent Tube Assembly to the Pump Holder

4 Connect the solvent tubing of the DLW Tubing kit to the pump module, using the premounted nut. Maintain this order: Solvent 1 connected to the left, and Solvent 2 to the right of the pump module, when viewed from the front of the system. This avoids any confusion when refilling or changing solvents.

5 Connect the solvent lines from the solvent reservoir bottle to the “Inlet” connectors of the pump module.

6 The electrical connections from the pumps to the PCB sockets, mounted on the bracket, are done at the factory.

7 Insert the cable provided into the middle socket, between the other two sockets on the PCB. For details see Figure 19 on page 70.

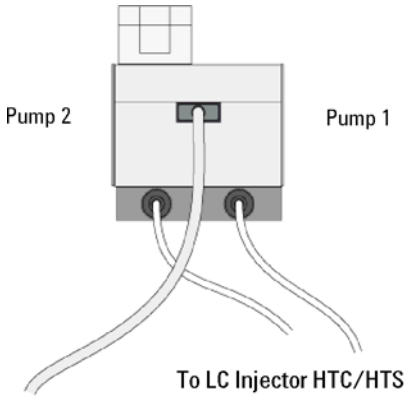


Figure 19 Electrical Connections at the Pump Holder Bracket

8 Connect the other end of the cable to the “Was Station” connector on the Control board, as shown in Figure 20 on page 70.

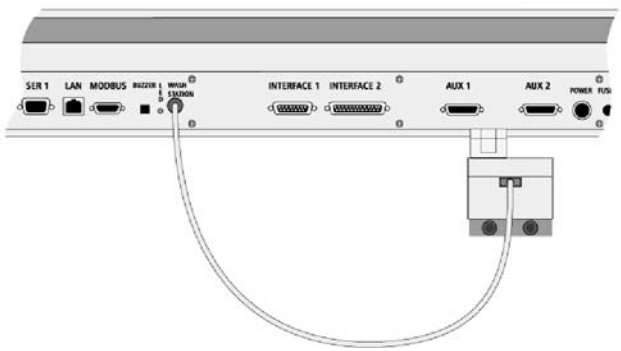


Figure 20 Electrical Connection from DLW Pump Holder to the LC Injector HTC/HTS Board

Installing the DLW Wash Station

The DLW Wash Station can be connected anywhere along the X-axis; however, it is strongly recommended to install it as close as possible to the Injection Valve. The travel path of the Injection Unit from the valve to the Wash Station should be as short as possible to keep the cycle time as brief as possible.

Figure 21 on page 71 shows two configurations for installing DLW modules, favoring a short connection from the injection valve to the other HPLC system components on the one hand, and always having the DLW Wash station close to the injection valve on the other. It is important that the DLW Pump Module, with the connected DLW tubing from the kit, always be attached on the far left side of the X-axis. Such a configuration also has the advantage that the waste tube from the injection valve can be connected to the front “Waste Inlet” of the Wash Station module. This waste tube is part of the DLW Option Kit.

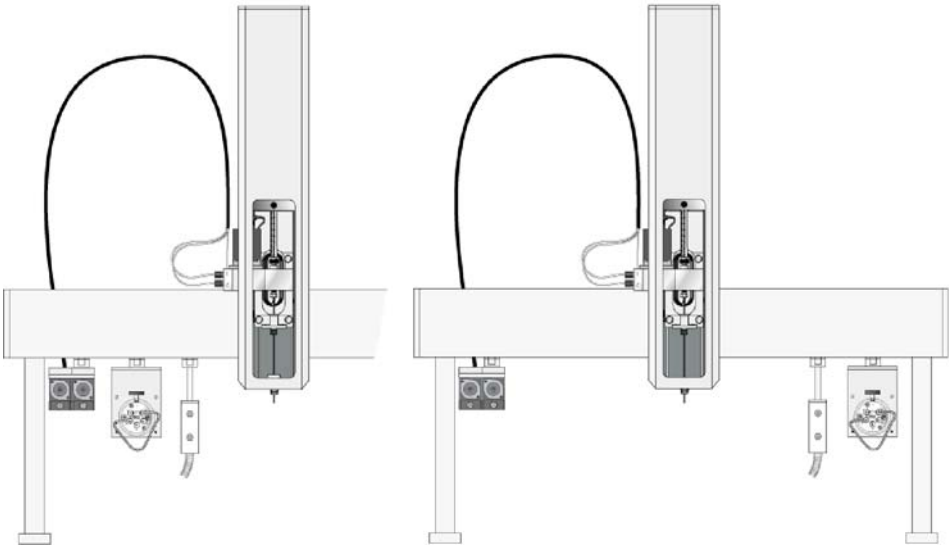


Figure 21 Mounting the DLW-2 Option Modules in combination with the Injection Valve

1 Locate the DLW Wash Station module.

2 Attach the holder to the X-axis at selected position as shown in Figure 21 on page 71.

3 Connect the Waste Tube from the Injection Valve to the DLW Wash Station.

4 Connect the Waste Tube to the Wash Station Waste Adapter.

5 Replace the dummy plug(s) on the front if used to connect the waste line coming from the injection valve.

   This sequence of waste lines simplifies the system, avoiding several waste lines running to the waste container.

NOTE

The other two dummy plugs underneath of the waste block are used for the DLW-2 option.

The DLW tubing from the DLW Pump outlet could be connected at these wash station inlet ports. The functionality of the Fast Wash Station would then be configured using this order of connections.

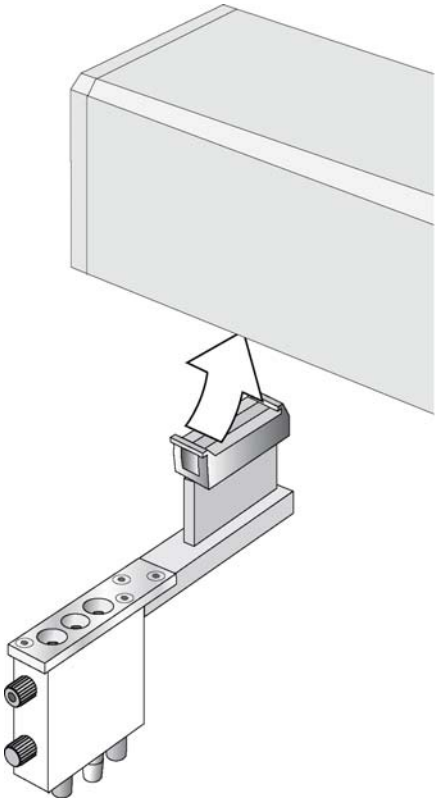


Figure 22 Attaching the DLW Wash Station to the X-Axis

NOTE The waste tube must be positioned below the injection valve. Make sure that the waste liquid flows into the waste container without any restriction. The waste tube must always be placed above the liquid level in the waste container.

Installing the DLW Syringe Holder Assembly

The DLW Syringe Holder differs from standard liquid syringe holders because the DLW Manifold is attached to the syringe holder. The DLW Actuator/Solenoid and the Holding Loop connecting the syringe inlet (bottom) and Syringe Needle are attached to the DLW Manifold. All of these parts are preinstalled at the factory. See Figure 23 on page 74.

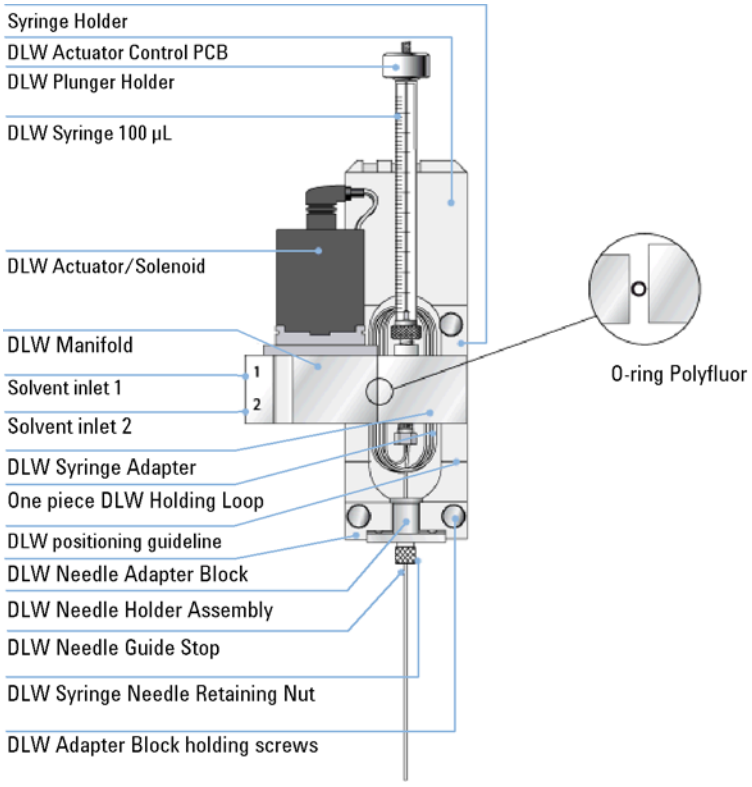


Figure 23 Front View of the DLW Syringe Holder Assembly

CAUTION

Needle damage

➔ Before a syringe is inserted, the position '**Change Syr**' should first be verified. The position for 'Change Syringe' should not occur above an object where the needle could collide with a vial.

➔ Path:

➔ **Menu > Utilitites > Syringe 'F3' 'Change Pos**'.

➔ Move the Injection Unit to a location where no collision can occur with the Z-axis by selecting the X- and Y- axes appropriately. The value for the Z-axis is given as a default and a change of this position is not necessary in standard operation.

➔ This precaution helps avoid needle damage during routine operation. Nevertheless, the description below on how to install the DLW Syringe Assembly recommends switching off the unit and inserting the assembly without using the command 'Change Syringe'.

Preparing and Installing the DLW Syringe

1 Prepare the DLW Syringe by inserting the DLW Plunger Holder.

   a First, move the plunger manually down to the stop position.

   b Release a slight amount of pressure from the plunger tip by pulling a fraction of a mm backwards.

   c Install the DLW Plunger Holder and tighten the Allen screw (Allen Key #6) firmly.

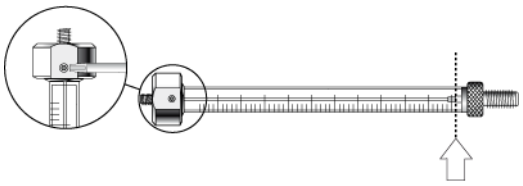


Figure 24 Inserting DLW Plunger Holder on the DLW Syringe

NOTE It is critical that syringes are primed before beginning sample preparation. Prime every liquid syringe first manually before inserting into the system.

2 Screw the prepared DLW syringe into the holder. Hold the syringe at the lower metal mount while tightening the syringe.

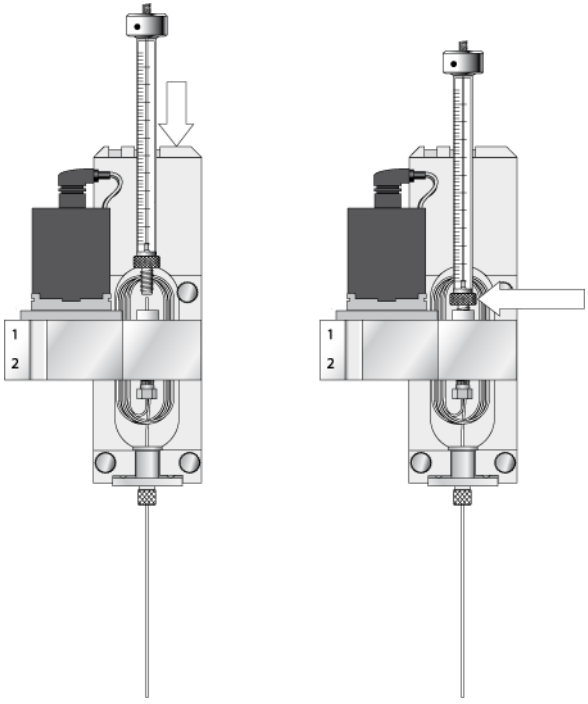


Figure 25 Inserting the DLW Syringe into the Holder

NOTE Holding the glass barrel while tightening can damage the seal where the glass meets the metal.

Connecting the DLW Tubing to the Syringe Holder

NOTE It is recommended to connect the DLW Tubing from the kit to the Syringe Holder while the assembly is not yet inserted in the X-axis.

NOTE The wetted parts of the DLW Actuator/Solenoid are composed of PEEK for the body and FFKM (Simriz) for the seal material. PEEK exhibits excellent chemical resistance to most commonly used chemicals. However, the following solvents are not recommended for use with PEEK: DMSO, THF, methylene chloride (dichloromethane), nitric acid, sulfuric acid. For more details, see the compatibility tables provided by the manufacturer of PEEK materials or components.

1 As described under "Mounting the Pump Holder" on page 68 the end of the the DLW kit sleeved tubing with the short tubes has to be connected to the DLW Syringe Holder Assembly.

2 Use the groove in the block at the back side of the DLW Manifold to orient the guide wire. Use the M3x5 screw provided and the corresponding serrated lock washer to connect.

3 Test if the wire tension is enough to keep the tubing in an upright position but low enough to move the syringe holder manually along the X-axis.

NOTE The design of the DLW Manifold and the concept of washing by active pumping and closing of the lines by the DLW Actuator theoretically does not require a predefined solvent line position, in theory. Problems could occur if solvent is refilled or exchanged while the tubing still contains solvent from the previous setup. Always keep the lines at the same position, and careful prime the entire system to prevent unnecessary confusion. See also "DLW Principles and Operating Details" on page 200.

4 Connect solvent line 1 to the upper port and solvent line 2 to the lower port at the left side of the DLW Manifold.

NOTE The order of the tube connections, upper and lower positions should be consistent.

NOTE For certain applications it is crucial not to mix types of solvents, for example biofluid sample solutions should not come in contact with highly concentrated organic solvents.

Inserting the DLW Syringe Holder Assembly into the Injection Unit

NOTE It is recommended to insert the DLW Syringe Holder assembly when the LC Injector HTC/HTS is powered off.

1 Move the Injection Unit aside manually to allow free movement of the syringe slider. Lower the syringe slider as shown in Figure 26 on page 79 in order to gain space for installing the DLW Syringe assembly.

NOTE Match the magnetic pins of the syringe holder using the counter positions on the syringe slider.

The position of the Guide Pin Holes can be matched with the 'Positioning DLW Guide Line' which is marked on the DLW syringe plate.

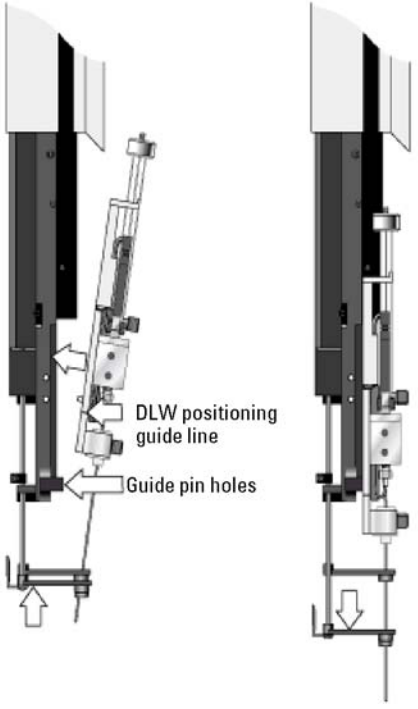


Figure 26 Inserting the DLW Syringe Holder Assembly into the Injection Unit

2 Press the syringe holder firmly against the Z-axis slider to ensure that the holder clicks into place.

HINT Do not press against the DLW Actuator/Solenoid. Its mounting to the DLW Manifold is fragile.

3 Tighten the knurled screw to fix the Holder Assembly to the syringe slider. (See Figure 27 on page 80.)

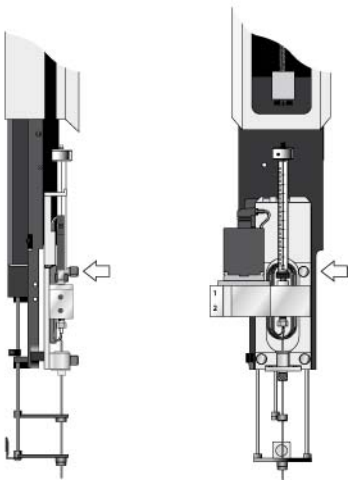


Figure 27 Fixing the Syringe Holder to the Syringe Slider

4 Move the plunger up (plunger holder) until the thread of the screw catches the thread of the plunger bushing. Tighten the screw to fix the plunger holder. (See Figure 28 on page 81.)

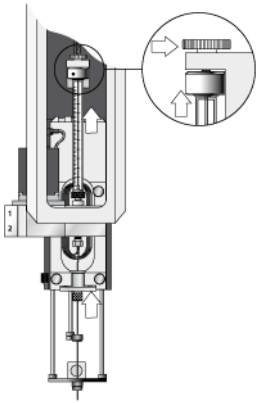


Figure 28 Connecting the DLW Syringe Plunger Holder

5 Tighten the holding screw to secure the syringe holder position.

6 Move the lower needle guide carefully up and down to make sure that the needle tip does not catch on the guide.

Injection Valve Plumbing and Connection to DLW Wash Station

1 Connect the injection valve Waste Line from port 6 for the Agilent 1200 bar LC injection valve 5067-4123, as shown in Figure 29 on page 82, to the front Waste Port of the Wash Station

NOTE If two injection valves are used in the LC Injector HTC/HTS configuration, the second waste line can be connected to the lower front waste port of the DLW Wash Station. If only one connector is used, apply a dummy plug to the second port on the Wash Station.

2 Place the Waste Tube from the DLW Wash Station into the waste container.

NOTE The waste container must be positioned below the injection valve. Make sure that the waste liquid flows into the waste container without restriction.

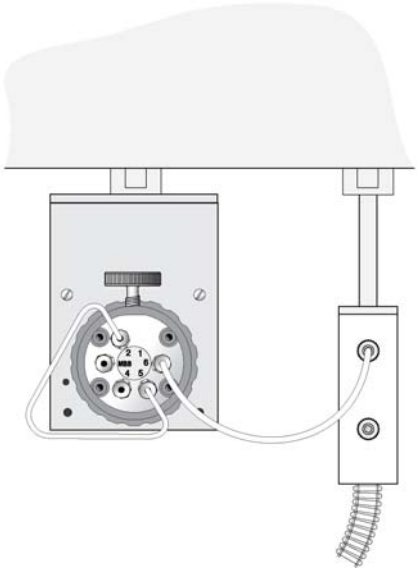


Figure 29 Solvent Line Plumbing on Rheodyne Valve PD Series

NOTE The Agilent specific Rheodyne Valve with Agilent PNo. 5067-4123 is connected in the same way to the HPLC system as shown in "Installation" on page 108. For details see the specific valve description.

Electrical Connections for the Single Injection Valve Setup

NOTE Always switch the LC Injector HTC/HTS power supply OFF before connecting or disconnecting the LC Injector HTC/HTS DLW cable or any other LC Injector HTC/HTS accessory cables!

The electrical connection of the DLW option is identical for the PAL HTS and the HTC models. The same board is used for both models.

1 The schematic in Figure 30 on page 83 shows an installation with the valve drive connected to the 'AUX' interface. If a serial or multiposition valve drive is used, see the schematics for 2- or 4-injection valve combinations.

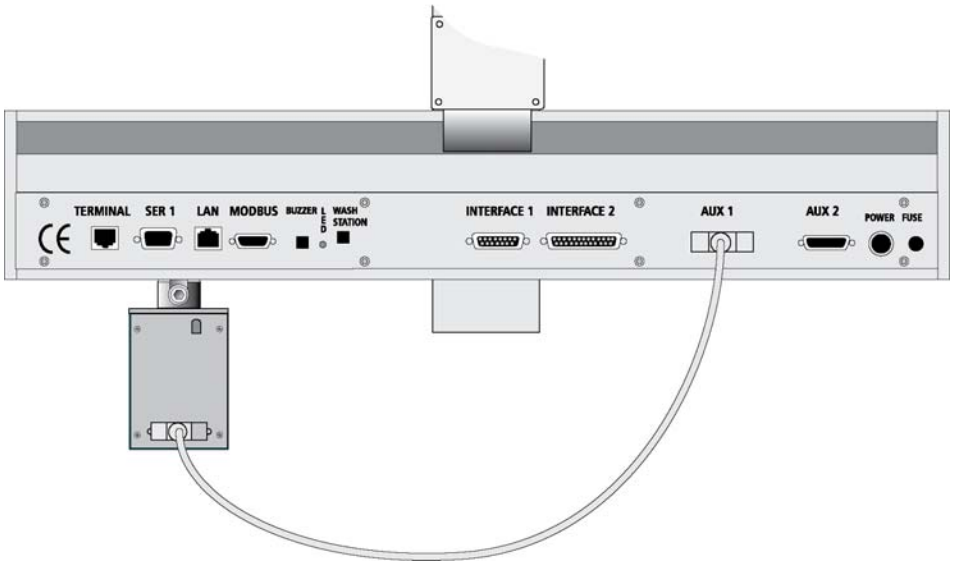


Figure 30 Electrical Connection, DLW Option on a PAL

Installing a Microplate Stack

1 If a Stack (a Tray Holder with multiple drawers) was shipped with the LC Injector HTC/HTS, loosen the two Torx screws on the two mounting clamps located on top of the Stack. The example below shows the two stack models with 3 and 6 drawers.

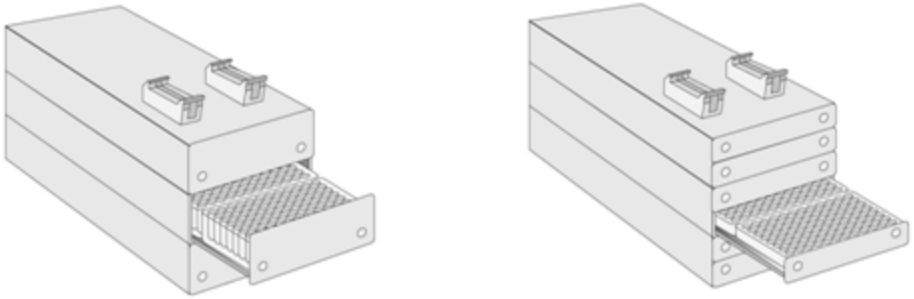


Figure 31 Installing a Microplate Stack with 3 or 6 Drawers

NOTE Installation of Stack and Tray Cooler is described in detail in the Addendum to the User Manual “MT/DW Tray, Stack, and Cooler Upgrades for Agilent LC Injectors HTC/HTS”.

2 Carefully lift the X-axis assembly on top of the Stack with the mounting clamp teeth fitting into the grooves on the bottom of the X-axis.

3 Be sure that the clamps fit completely into the grooves. Alternately tighten the two Torx screws until the two mounting clamps are firmly in place.

4 Double check whether the Stack clamps are correctly attached to the X-axis (see Figure 8 on page 55).

Installing a Trayholder

1 If a Tray Holder was shipped with the LC Injector HTC/HTS, loosen the two Torx screws on the two mounting clamps located on top of the Tray Holder legs.

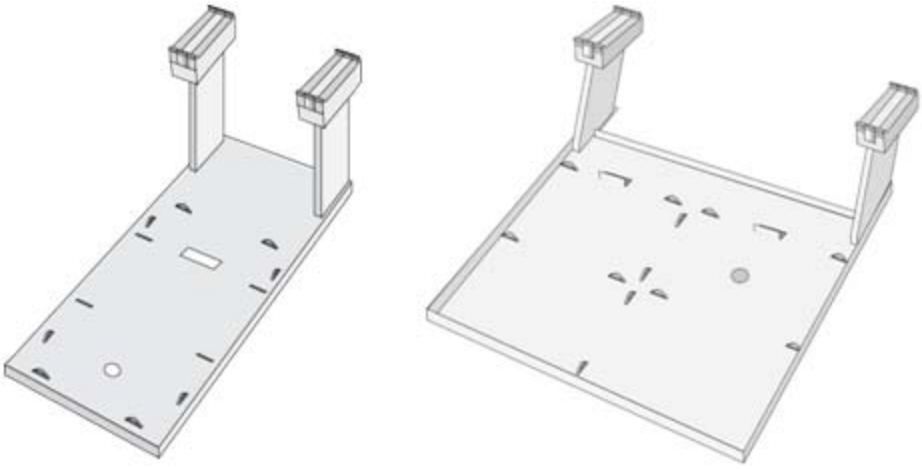


Figure 32 Installing a Tray Holder

2 Install the Tray Holder with the mounting clamp teeth fitting into the grooves on the bottom of the X-axis.

3 Be sure that the clamps fit completely into the grooves. Alternately tighten the two Torx screws until the two mounting clamps are firmly in place.

4 Double check whether the two Tray Holder clamps are correctly attached to the X-axis (see Figure 8 on page 55).

Electrical Connections

NOTE Always switch OFF the power supply before connecting or disconnecting any accessories cable!

NOTE Do not interchange the Terminal with LAN cable connectors.

1 Before defining the object positions, make sure the LC Injection Valve, Fast Wash Station and Keypad Terminal are correctly connected to the X-axis rear side (see Figure 33 on page 86 or Figure 34 on page 87).

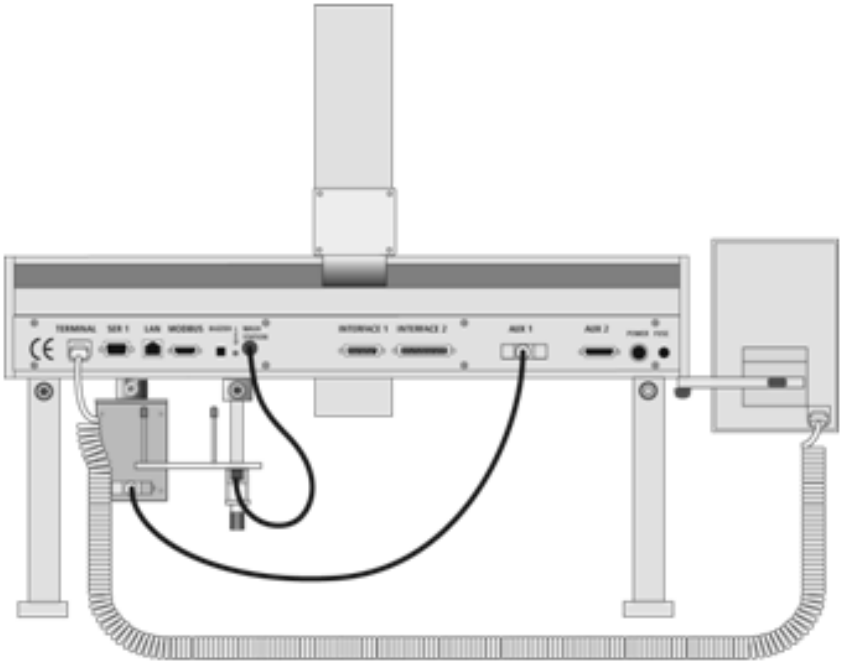


Figure 33 Electrical Connections - LC Injectors HTC

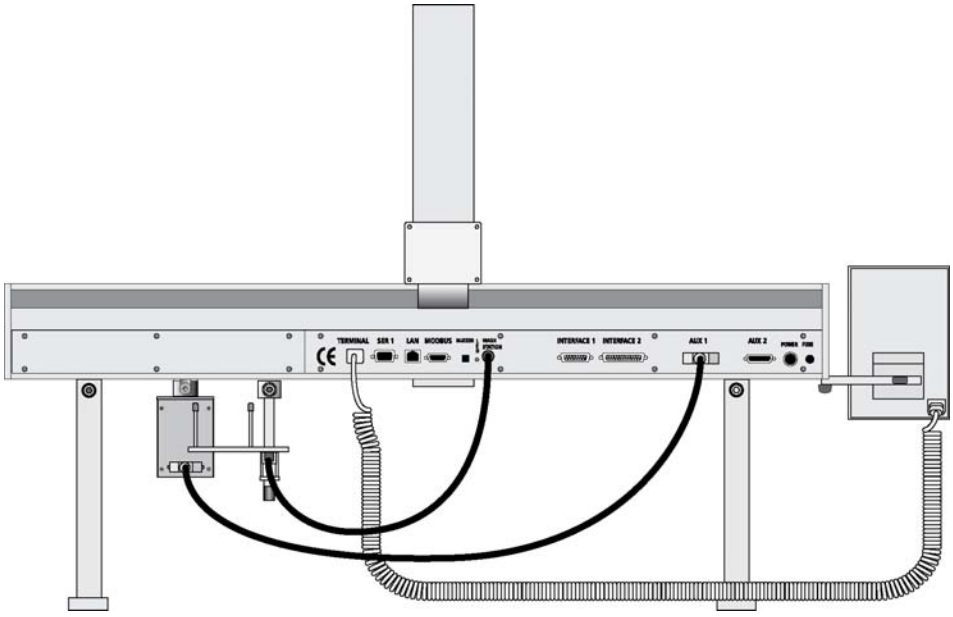


Figure 34 Electrical Connections - LC Injector HTS

Object Positions

Defining Object Reference Positions

The objective is to define the Reference Positions for all autosampler Objects. Make sure the Tray Holder(s), Valve Drive(-s)and Wash Station are properly mounted to the autosampler X-axis. The following description is an example of how to teach the reference position for a Tray Holder. The described procedure is common to all autosampler Objects.

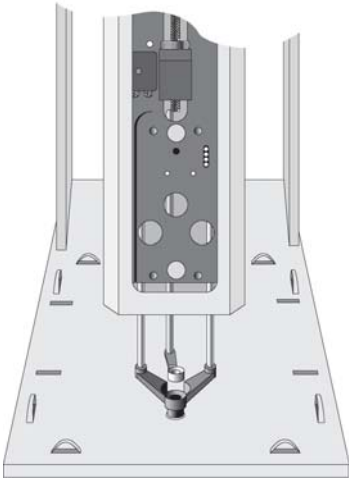


Figure 35 Object Reference Position

NOTE Remove the Syringe Adapter from the Injection Unit before performing the following steps.

For a Tray Holder, the *Reference Position*is a hole (slightly larger than the lower needle guide) in the base plate of the holder. The lower needle guide should be centered in the hole with the bottom of the needle guide flush with the bottom of the base plate (see Figure 35 on page 88).

1 Switch ON the autosampler power supply.

2 Observe the keypad display. The model name will display along with the software version number. The **Job Queue** menu screen will then display.

3 When the "**Job Queue**" menu displays, complete the following sequence (common to all objects):

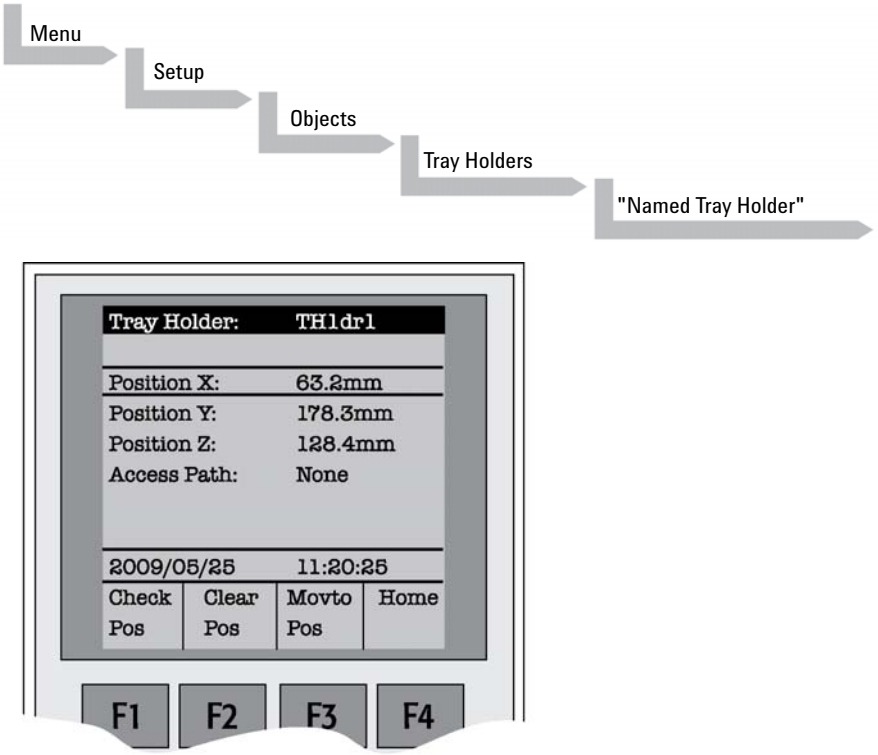


Figure 36 Menu Screen Object Trayholder

1 "**Named Tray Holder**" represents a predefined Tray Holder, (e.g. THldr1 or Stack1).

4 After selecting "**Named Tray Holder**" the *X- Y- Z*-positions for the object will be displayed (see Figure 36 on page 89).

5 Highlight item **Position X** with the cursor bar and press **ENTER**. The Injection Unit will move to the previously defined X-axis position.

6 Rotate the outer knob to adjust the X-axis position to the Tray Holder reference position.

7 Press the inner knob to *ENTER* the **Position X** value.

8 Repeat steps 5-7 for **Position Y** and **Position Z**.

9 If **F3** "**Moveto Zero**" is activated, the Injection Unit will move to the **HOME** position;

10 Verify the defined **X-, Y-, Z-positions** by pressing **F1** "**Check Pos**".

Figure 37 on page 90 illustrates possibilities of correction. Details for teaching are given below.

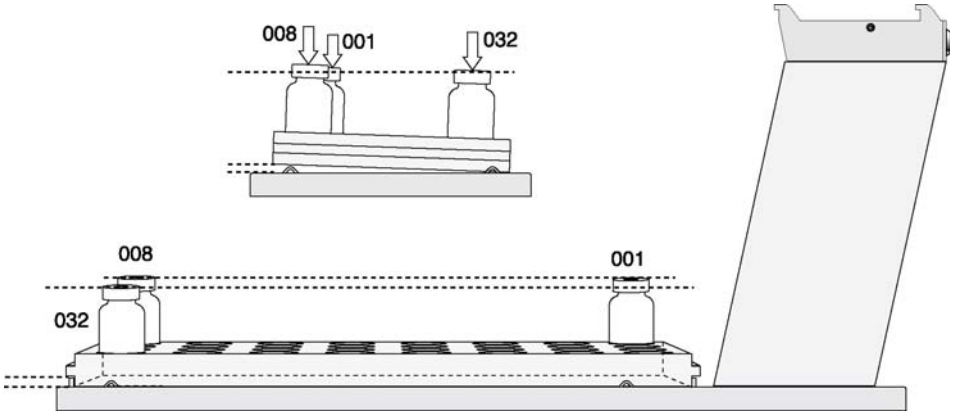


Figure 37 Inclined Tray, Corrections for X-, Y-, Z-Axes

NOTE The release of Firmware version 4.1.X makes possible the correction of all three axes, X-,Y-, Z, in the firmware class " **Tray**." The correction of the inclination can be made for a tilted Tray in the direction of the row and/or the column. Teaching is possible in the " **Utilities**" section by using the path: Menu/Utilities/Trays.

NOTE These functions are not available for the autosampler if operated solely on PAL Firmware version 2.4.X or higher, but neither with firmware version 3.X.X. nor with version 4.X.X.

Description of Object Reference Positions

Trayholder (e.g. THldr1)

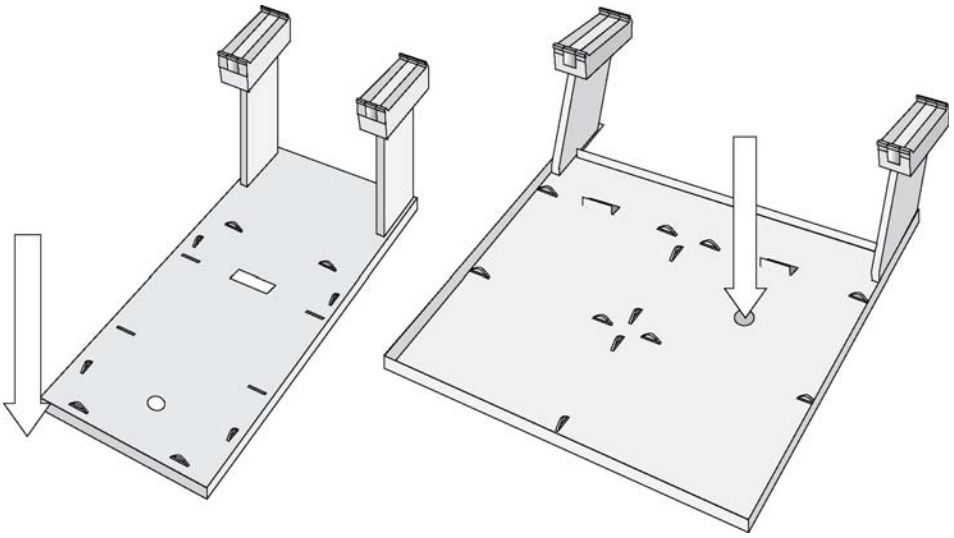


Figure 38 Tray Holder Reference Position

For Tray Holder, the *Reference Position* is a hole in the base plate of the Holder. The lower needle guide should be centered in the hole with the bottom of the needle guide flush with the bottom of the base plate.

Figure 38 on page 91 shows the example of a Tray Holder for a single Tray (e.g. “THldr1” for “Tray 1”) and a Tray Holder for four Trays (e.g. “Tray 1” with Tray Type “VT54”, “DW96” or “MT96”, etc.).

Tray Holders Stack or Stack Cooler with 3 or 6 Drawers

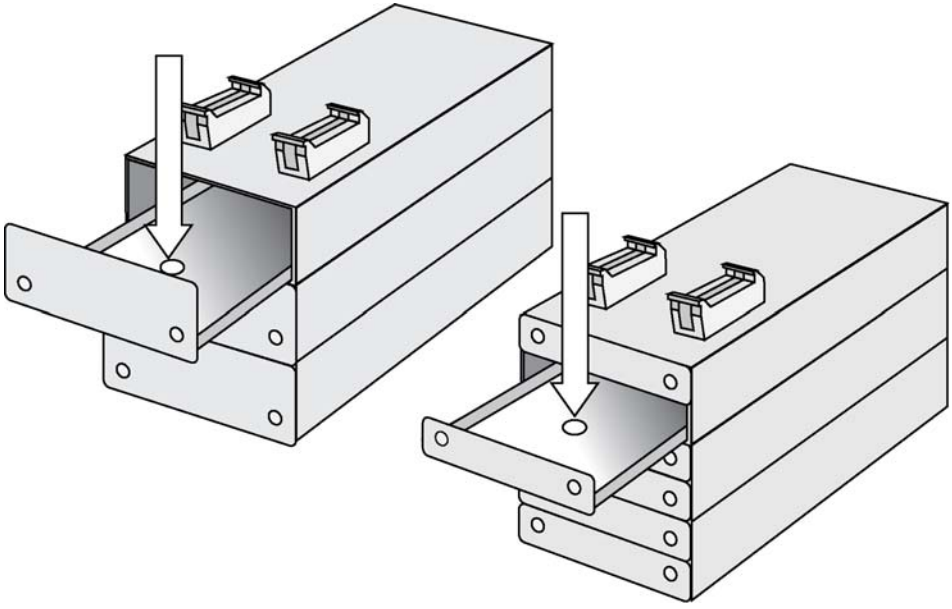


Figure 39 Stack Reference Position

The *Reference Position* for a Stack or Stack Cooler is identical. The reference position in the case of a 3-Drawer Stack is a hole in the upper drawer and in a 6-Drawer Stack in the second drawer from the top (same height level), as shown in Figure 39 on page 92.

Trays

In general it is not necessary to teach a Tray position. The Tray Holder has a teaching position, as described above. A Tray is assigned to the Tray Holder. The numbers (values) for the relevant items are stored in the firmware object “Tray”. To complete the combination of a Tray Holder and a Tray, it is necessary to assign the corresponding “Tray Type” to the “Tray.” The “Tray Type” contains the geometric data for the Tray and information, such as how many samples are in a row and a column in the Tray. A Tray Type defines the pattern and sampling sequence of sample location within a Tray. For details, see below.

As described above, the Tray is physically placed in the Tray Holder, the firmware assigns the Tray to the Tray Holder and the Tray Type to the Tray. This is routine procedure as long as no special circumstances need be considered, such as customized Trays or Tray Types.

Autosampler Firmware version 4.1.X now makes a correction possible if the Tray surface is not exactly horizontal and planar but is inclined in one or more axes. At version 4.1.X this is standard and a dialog window pops up the moment the Tray has been checked-out at the corners to verify vial positions and heights. How to correct a possible inclination is explained below.

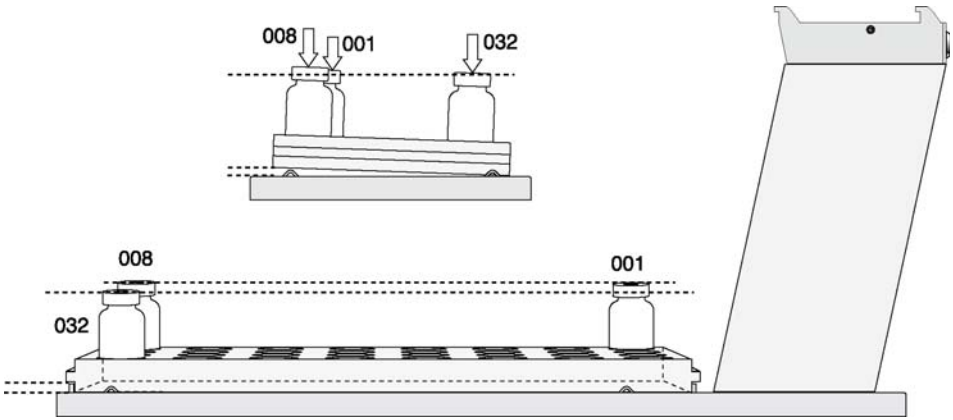


Figure 40 Demonstrating a possible Inclination of Tray in X-, Y-, Z-Axes.

Definition of a Tray Row and Column

The autosampler system defines Rows and Columns by the order in which samples are treated. A “Row” is not associated with an X- or Y-axis. The definition is visualized below with Tray Type VT32 and DW96:

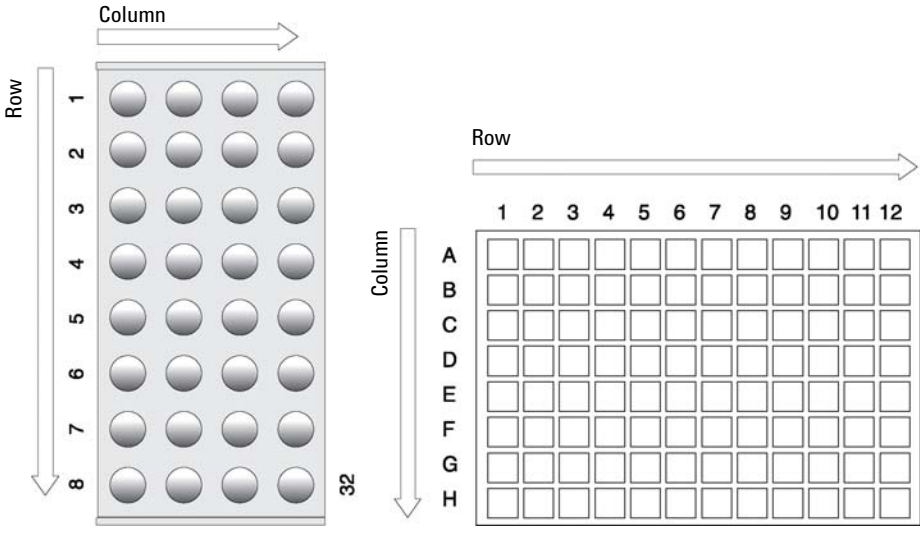
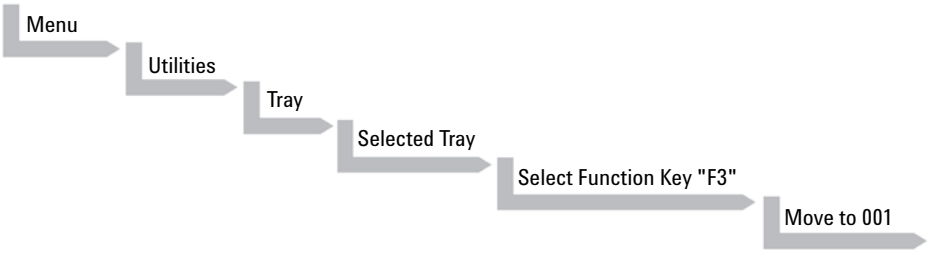


Figure 41 Tray Type VT32 and Tray Type DW96

Correction for Sample Position

The following description of the correction is done by using the path to the "**Utilities**" section.

After selecting the Utilities function "**Trays**", verify whether the Tray Type assigned corresponds to the Tray placed into the Tray Holder. If this is verified, the vial positions can be checked at the three corners of the Tray. This is activated by the "**F3**" function key.



A dialog window opens, displaying a note to "**Adjust offset X-, Y-, Z for Position 001**". Continuing in this dialog will provide the user the opportunity to adjust all three axes perfectly by the top of the vial. Please note that this step is only valid for position number 001.

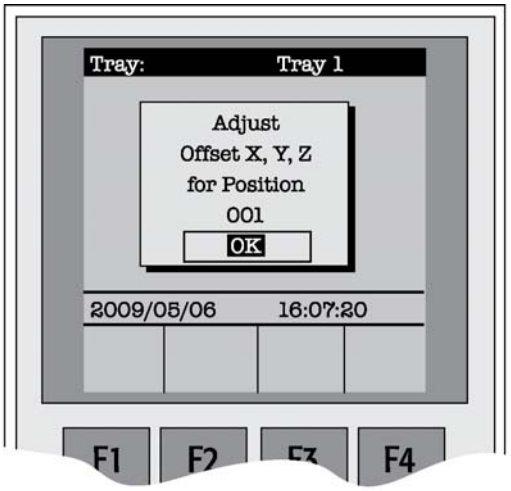


Figure 42 Adjusting "**Offset X,Y,Z**" for Tray Position 001

To check the next corner of the Tray, activate function key “**F3**” again, example Tray Type “**VT32**”, position 008.

The next information provided by the pop-up window asks to correct a possible inclination of the “**Row**”. Continuing the dialog provides the user the opportunity to adjust the three axes perfectly to match the vial top – or in case of a well-plate, the top of the plate.

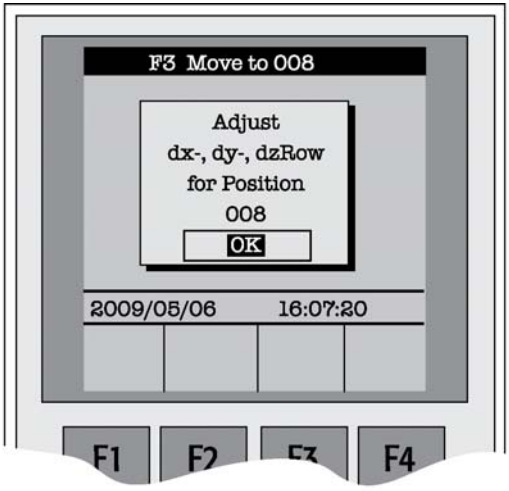


Figure 43 Adjusting Row Inclination at second Tray corner position

The last step will be to move to the next corner of the Tray by again activating function key “**F3**”. This will be position 32 in the example of Tray Type “**VT32**”.

A possible inclination of the Tray column can be corrected in the same manner as described above.

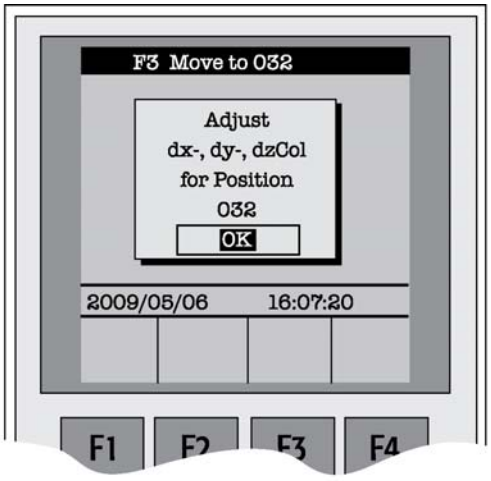


Figure 44 Adjusting Column Inclination at Third Tray Corner Position

The three corner points are now adjusted for a possible inclination of the Tray in any axis. A possible deviation from an ideal axis position for the other vials, caused by variance from the horizontal, is interpolated by the system without further teaching.

NOTE For routine work using standard vials it is not necessary to compensate for a possible inclination as long as the vial detection is within the "**Z-Tolerance**" range.

Loading the LC Injector HTC/HTS DLW Object

If the version of the PAL Object Manager List installed on the computer is not '**PAL-xt Object Lists Rev. C**', copy the provided folder 'Wash Station Option' (See Figure 45 on page 99.) from the CD-ROM to the Object Lists folder which has been installed with the Object Manager. This software is usually installed in the following path:

C:\Program Files\PAL\Object Manager\Object Lists

Using **PAL-xt Object List Rev. C** or higher, the DLW Option object will become a part of the list in the folder '**Wash Stations**' folder. The following Object List is then available:

PAL DLW: DLW Option Firmware Object List

NOTE The instructions in the following section have to be followed for upgrades only. All Agilent 1290 Infinity LC Injector HTC/HTS are configured with the right objetcs from the factory.

1 Open the Object Manager and verify the revision number of the Object Lists.

   If **PAL-xt Object Lists Revision level 'C'** (or higher) is available, continue as described below.

   If a '**PAL Object Lists**' version is used (for example Rev. K or L), copy the provided DLW Object List into the Object Manager folder as described above.

2 Select the '**PAL DLW**' Object list and click '**Send selected Object Lists to PAL**'.

3 Close the Object Manager.

The DLW Object list contains the following LC Injector HTC/HTS Firmware Objects which are loaded with a single command as described above:

Table 27 DLW Object List

Object Class	Object Name
Motor Drive	MPIgMed
Heater	HDLW
Syringe	100uIDLW
Wash Station	Wash1
	Wash2
Injector	Waste
PWR Event	Pwr-Out1
	Pwr-Out2

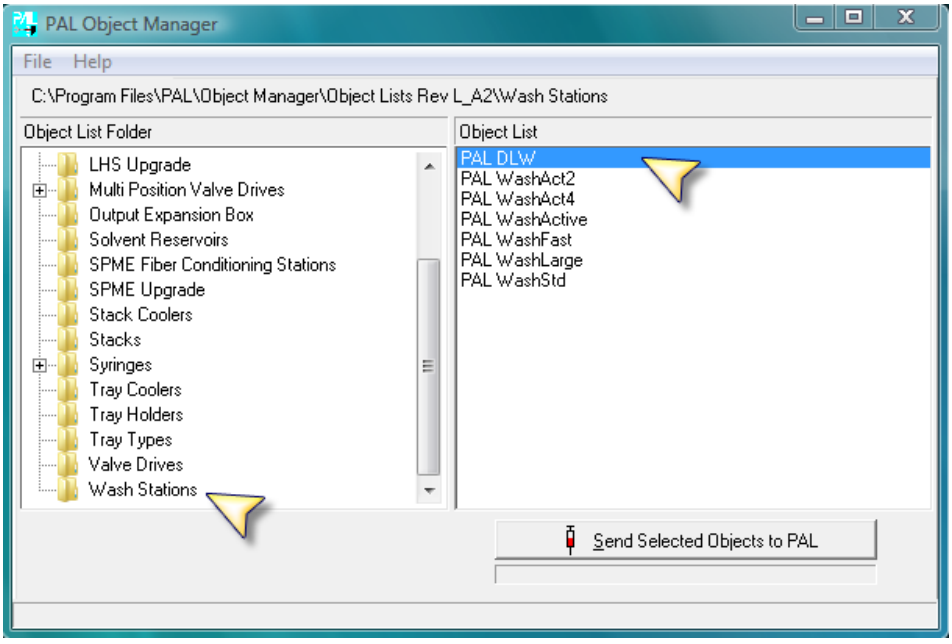


Figure 45 Object Manager; choosing Object List Folder '**Wash Stations**'

NOTE For more details on the PAL Object Manager software, see the Addendum to the PAL User Manual *PAL Object Manager Software*.

Wash Station Reference Points

Wash1/Wash2 Reference Points

For the DLW Wash Station Option, the reference positions for '**Wash1**' and '**Wash2**' are the two holes on top of the Wash Station assembly. See Figure 46 on page 100.

The lower needle guide should be centered on these holes with the bottom of the lower needle guide lightly touching the surface of the Wash Station assembly.

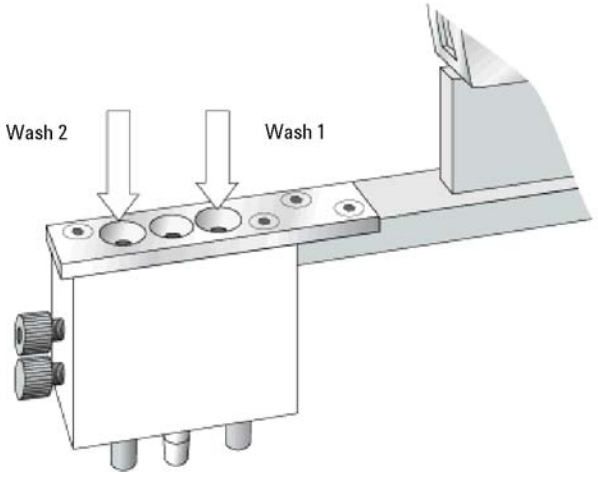


Figure 46 DLW Wash Station Wash1/Wash2 Reference Points

1. Press '**HOME**' on the handheld controller and select the sequence **Menu > Setup > Objects > Wash Stations > Wash1**.
2. Enter Wash1 and adjust the X-, Y-, Z-positions until the lower needle guide of the Injection Unit centers on top of the Wash station assembly. Fine tune the Z-position until the bottom of the lower needle guide is flush with the top surface.
3. Repeat steps 1 – 3 for **Wash2** by using the path: **Menu > Setup > Objects > Wash Stations > Wash2**

Waste Reference Point

The reference position for the *Waste Port* – a hole (slightly larger than the needle guide) – has been placed in the middle positions of '**Wash1**' and '**Wash2**'. (See Figure 47 on page 101.) The lower needle guide should be centered on this hole with the bottom of the lower needle guide lightly touching the surface of the Wash Station assembly.

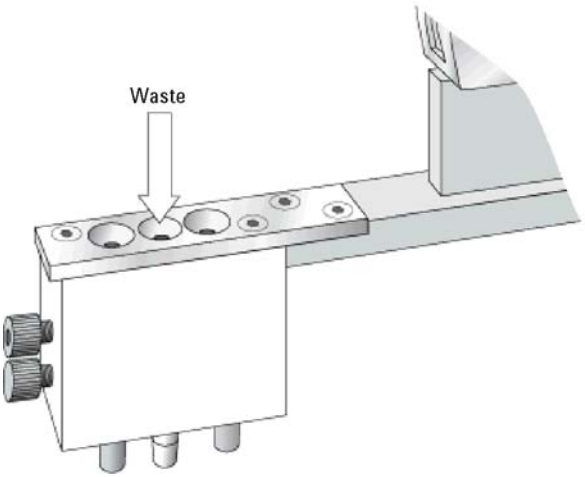


Figure 47 Waste Reference Position

NOTE The Waste position is defined as an '**Injector**' by the software. It is classified in the Object category '**Injectors**'.

1. Using the handheld controller select the sequence: **Menu > Setup > Objects > Injectors > Waste 1**.
2. Enter Waste and adjust the X-, Y-, Z-positions until the lower needle guide of the Injection Unit centers on top of the Wash Station assembly. Fine tune the Z-Position until the bottom of the lower needle guide is flush with the top surface.
3. Press '**F1**' to verify the position and '**F4**' for '**Home**'.

Injection Valve Reference Points

For an LC Valve, the reference position is the valve needle guide fitting mounted on the top valve port (see Figure 48 on page 102).

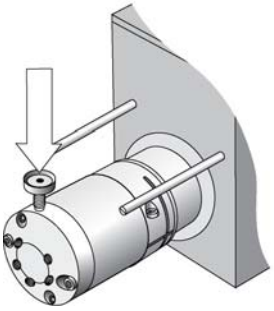


Figure 48 LC-Valve Reference Point

The lower needle guide of the Injection Unit is centered on the valve needle guide fitting. Adjust the Z-Position so that the bottom of the lower needle guide just touches the surface of the valve needle guide fitting. *Then reduce this value by 2.0 mm* (see Figure 49 on page 102).

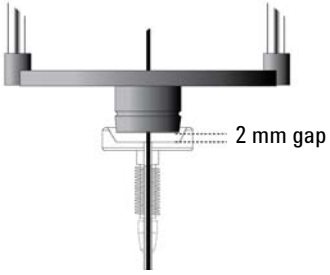


Figure 49 Positioning the Z-Axis Needle Guide on the Valve Needle Guide

NOTE To adjust the Valve Needle penetration value, see "Defining the Valve Penetration Value" on page 103.

Defining the Valve Penetration Value

NOTE Before performing the following steps, make sure that Object positions X, Y, and Z for LC Vlv1 are properly defined and the complete DLW Syringe assembly, is inserted into the injection unit.

Complete the following steps to define the Valve Needle Penetration Depth:

1. Using the handheld controller select the sequence: **Menu > Setup > Objects > Injectors > LC Vlv1**
2. Press **F1** '**Check Pos**'. The Injection Unit moves to the previously defined LC Vlv1position.
3. Highlight the item '**Needle Penetr**' with the cursor bars by pressing **ENTER**.
4. Slowly rotate the outer knob to adjust the needle penetration depth.

   The syringe moves down stepwise into the injection port.
5. When the syringe needle tip enters the valve needle guide, slow down the Z-movement again.

NOTE Always observe the syringe needle during this adjustment step.

6. Move down stepwise until the bottom of the cross bar of the DLW Needle Holder Assembly is flush with the lower line of the 'DLW Needle Adapter Block' (see Figure 50 on page 104).
7. Press **ENTER** to save the needle penetration depth value.
8. Verify the defined Needle Penetration Depth value by repeating steps step 3 on page 103 to step 6 on page 103.

NOTE If a 'click' sound occurs, this means the ball-spring loaded '**Needle Holder**' is completely compressed. **STOP** the Z-axis downwards movement immediately. Rotate the outer knob stepwise in the opposite direction until the gap in the spring loaded "**Needle Holder**" is approximately 1 mm, or the bottom of the '**Holder Assembly**' is flush with the lower surface of the '**Needle Adapter Block**', as in Figure 50 on page 104. Repeat the needle penetration test and verify the needle penetration depth.

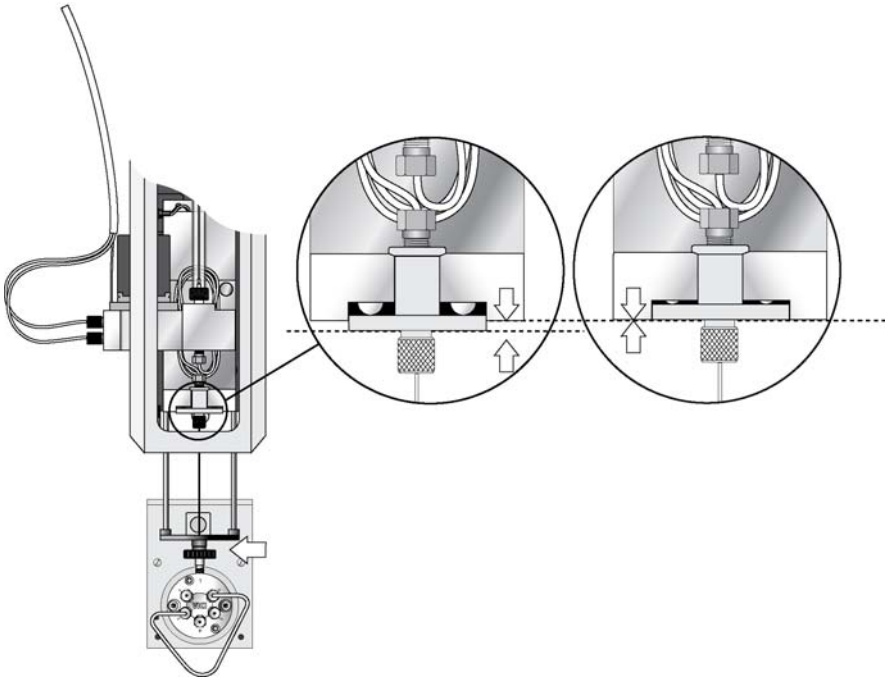


Figure 50 Teaching the Needle Penetration Depth Value

An example of the correct and incorrect positioning of the syringe needle tip in the valve inlet port is shown in Figure 51 on page 104.

Figure 51 Correct and incorrect Valve Needle Penetration Depth

The ball-spring loaded 'Needle Holder' presses the square cut needle tip firmly against the bottom of the valve body. This ensures a constant seal during the injection process Figure 50 on page 104.

Injection Valve

Valve Drives and Valves – General Remarks

A LC Injector HTC can be equipped with 1 LC Injection valve connected and controlled via the Auxiliary Interface (AUX). A LC Injector HTS can be equipped with 1 or 2 LC Injection valves connected and controlled via the Auxilliary Interface (AUX1, AUX2). If more than one valve has to be configured, or if an auxiliary Interface is occupied by another module, such as a Dilutor, then Multi Position or Serial Valve Drives are required.

NOTE The LC Injector HTC is equipped with the control board "APR Control-xt" which provides two auxiliary interfaces, "AUX1" and "AUX2". The choice to connect one more valve drive is provided.

The standard LC injection valve is a six-port 1200 bar Rheodyne Injection valve.

The six-port valve is connected to the detection system according to the flow path shown below. Plumbing diagrams for other available six-port injection valves are provided below as well.

Details are described in the "Valve Drives and Valves – General Remarks" on page 105.

Injection Valve Flow Path

The Agilent Injection Valve 5067-4123 is a SupraLife™ 2-position, 6-port Rapid Replacement Pod equipped with a vertical inlet port, a pinned drive and designed for very high pressure applications. The concept is based on a flat plate rotary valve.

Plumbing Diagram

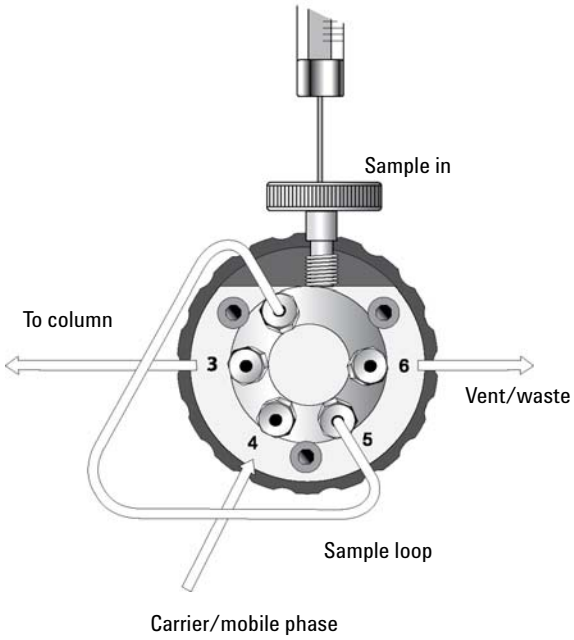


Figure 52 6-Port Agilent Injection Valve 5067-4123

Specifications

Table 28 Specifications

Valve Stator:	SPC-5 coated stainless stator
Rotor:	RPC-13 Polyimide based rotor seal
Bore Size:	Vertical Port: 0.25 mm (0.010 in) Ports 2 to 6: 0.20 mm (0.008 in)
Volume for Vertical Port:	0.75 µL
Ports and Engraving:	Ports 2 to 6: 0.07 µL Engraving: 0.05 µL (standard engraving)
Valve Volume Full Loop:	Full Loop: 0.87 µL Partial Loop: 0.82 µL
Connections:	10-32 male threaded fittings, 1/16 in tubing OD
Maximum Pressure:	1200 bar (18000 psi; 120 MPa)
Standard Loop:	2 µL; Loop ID: 0.13 mm (0.005 in)
RoHS:	Compliant

Installation

1 After the mechanical connection of the PAL Valve Drive to the X-axis and electrical connection to AUX1 at the board, turn on the power of the PAL System.

The Firmware Object for the Valve type **PAL VlvDrv-5067-4123_1** is preloaded, the Valve Drive will go into the Standby position.

2 Insert the valve body into the shaft until the pin engages in the shaft.

3 Center the valve body by turning the valve body into position until the vertical port is aligned.

4 Insert the Valve Body completely into the shaft until the pin on top of the valve engages in the threaded Valve Drive Bracket. Tighten the Valve Body with the polymer connecting nut.

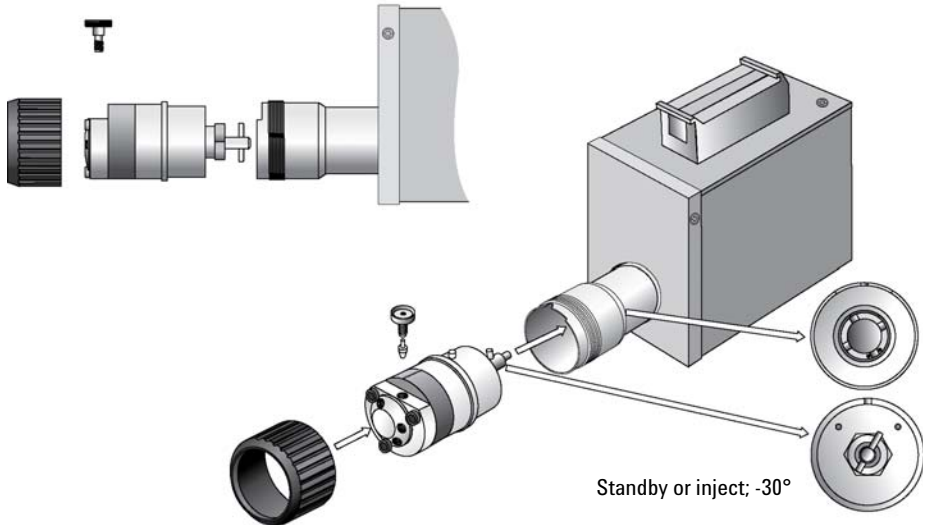


Figure 53 Valve Installation

Object Manager

Prerequisite for the LC Injector HTC/HTS models is Firmware level 4.1.X or higher, PAL Loader software version 2.1.0 (or higher) and the PAL Object Manager software version 2.2.1 (or higher) with PAL-xt Object Lists Rev. A.

PAL-xt Object list Parameters:

- Object Name: **PAL VlvDrv-5067-4123_1**
- Active: +30 °C
- Standby: -30 °C

Definitions:

- Active or Load: Fill sample Loop (Loop in low pressure position)
- Standby or Inject: Load sample from Loop to column (Loop in high pressure position)

Valve and Actuator Positions

Table 29 Rotor position, view from backside of valve body (actuator side).

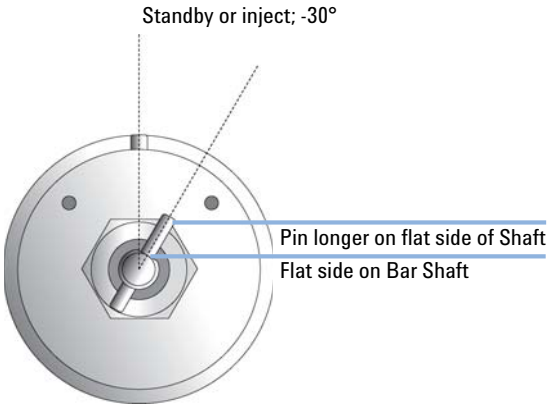


Figure 54 Valve position "**Standby**"

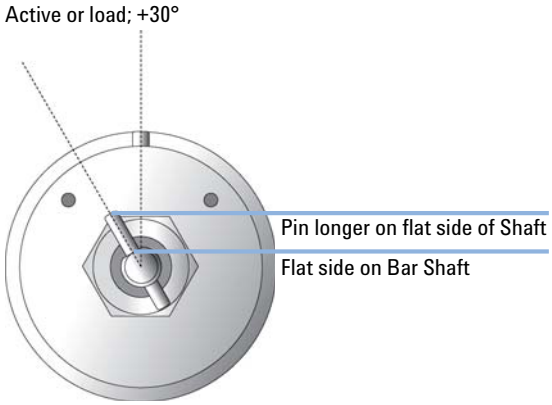


Figure 55 Valve position "**Active**"

Table 30 Valve Drive Actuator, View from front of Actuator.

Figure 56 Actuator Position "**Standby**"

Figure 57 Actuator Position "**Active**"

Spare Parts

p/n	Description
5067-4123	Valve (complete) 2 PS/6PT 1200 bar
5068-0029	Stator 2PS/6PT 1200 bar
5068-0030	Rotor Seal, 2PS/6PT 1200 bar
5188-6478	Needle Seals, Rheodyne Valve (10/pk) FEP tubing with SST ferrule, Needle Gauge 22 (OD 0.72 mm)
5068-0031	2 µL Loop max operation pressure 1200 bar
5068-0032	5 µL Loop max operation pressure 1000 bar
5068-0051	10 µL Loops max operating pressure 1000 bar
5068-0033	20 µL Loop max operating pressure 1000 bar
5068-0034	50 µL Loop max operating pressure 600 bar
5068-0035	100 µL Loop max operating pressure 600 bar

Valve Needle Guide

All valves are equipped with a special valve needle guide fitting. This fitting has a wide diameter to mate with the syringe needle guide on the injection unit. The valve needle guide also has a countersunk hole to facilitate insertion of the syringe needle into the injection port.

Figure 58 on page 112 shows the example of the setup with the standard valve, Cheminert type.

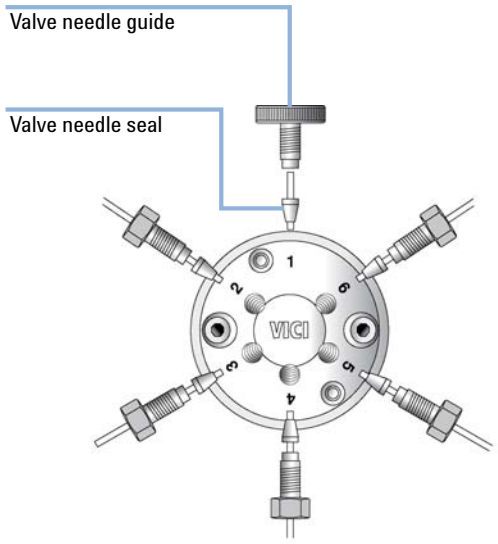


Figure 58 Valve Needle Guide and Valve Needle Seal

The valve needle guide holds the needle seal. The needle seal, which is a short length of FEP tubing, forms the seal around the syringe needle. A stainless steel ferrule is tightened around the FEP sleeve to ensure a leak-proof fit.

When

The interval to replace the Needle Seal depends on the number of penetrations. Two factors may be considered for the decision to replace the Needle seal:

- Leak, seal not reliable

  Remove the Needle Seal from the inlet port and insert a syringe manually with the corresponding needle gauge (standard Gauge 22 OD 0.72 mm). Move the needle below the seal point of the ferrule. If no resistance is observed at this point, replace the Needle seal.
- Carry-over

  In case of any doubt of possible contamination of the Needle Seal with a compound, replace the Needle Seal. Contamination can occur if the sample solution is accidentally dispensed too fast into the valve system. The restriction of valve and loop may cause backpressure and the sample solution can be forced backwards into the inlet port of the valve.

Parts required

#	p/n	Description
1	5188-6478	Needle Seals, Rheodyne Valve (10/pk) FEP tubing with SST ferrule, Needle Gauge 22 (OD 0.72 mm)

NOTE

LC Injector HTC/HTS Firmware (level 2.4.X or higher) provides a counter for the number of injections performed with the LC Injector HTC/HTS System. This counter monitors the valve switches and not the number of penetrations into the injection port, e.g. for cleaning the valve an extra penetration is required. It is advisable to monitor this counter in conjunction with the test for the tightness of the Needle Seal as described above.

- After running routinely a few weeks, the warning limit for the “Inject Counter” gradually becomes more evident and will be a valuable tool for the user in routine work.
- Path: **Menu > Info > Maintenance > Inject Limit** (“Inject Counts” reflects the actual number of injections).

NOTE

To ensure reproducible sample injection and minimize carry-over it is critical that:

- The valve needle guide and the valve needle seal be installed properly,
- The valve needle seal be changed on a regular interval, details see below,
- The needle penetration depth be set accurately.

To specify a limit number of maximum penetrations into the Needle Seal is not practicable. It is advised to check the tightness of the seal daily or weekly when first using the LC Injector HTC/HTS System to gain experience with the specific use in the laboratory related to the application.

The following simple test provides assurance of tightness:

1. Take the Needle Seal out of the valve port.
2. Manually insert a syringe needle gauge 22 (or Gauge 22S) from top down into the tube, passing the sealing point of the ferrule.
3. If a restriction is observed, continue to use the Needle seal. If not, replace the Needle Seal.

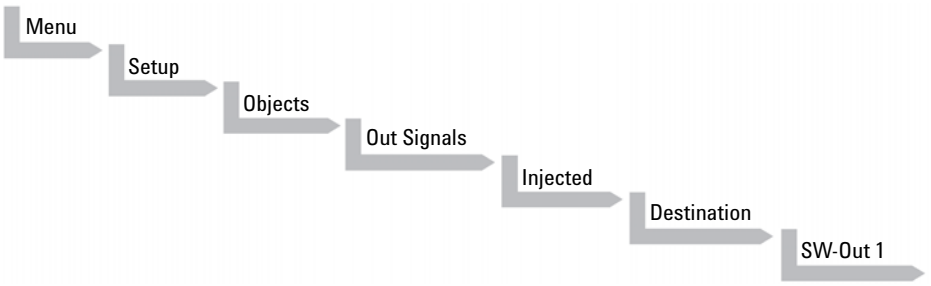
Interfacing the LC Injector HTC/HTS to other Devices

Synchronization and Output Signals

Synchronization Signals (**Sync Signals**) are inputs that tell the LC Injector HTC/HTS when to wait or proceed with a sample-processing step. Output Signals (**Out Signals**) are sent from the LC Injector HTC/HTS to external devices to indicate status or completion of particular processing steps. These signals are classified as Objects. Physical Events (e.g.TTL-In1) are also Objects and may be assigned to named signals. LC Injector HTC/HTS Cycles require that certain signals such as "**Start**", "**Inject**", and "**Injected**" be defined.

See Table 31 on page 116 for the LC Injector HTC/HTS Events and signal assignments associated with the standard LC Injector HTC/HTS "**LC-Inj**" Cycle.

Certain types of integration of the LC Injector HTC/HTS system into data handling or control software, such as ChemStation, Masshunter or EZChrom accomplish synchronization of the Ready/Start signal directly via software control (RS232/LAN). A synchronization cable is not required and the Sync Signal "**Start**" must be set to "**Immediat**".



To assign a physical Event (e.g. SW-Out 1) to an existing signal Object ("**Injected**") complete the following menu selections:

LC Injector HTC/HTS is shipped with all Cycle events pre-defined as shown in the table below. If a different physical signal needs to be used, then it will be necessary to assign a new Event to the Object signal.

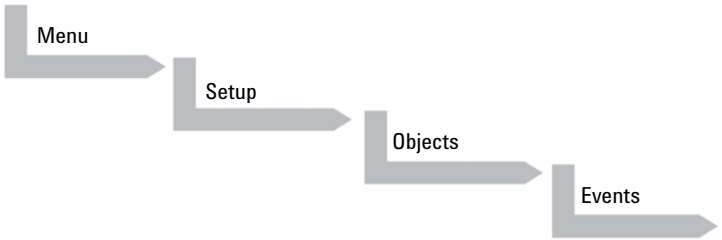
If a signal is to be ignored, then its corresponding Event must be set to "**Immediat**".

Table 31 Standard Assignment for LC Injector HTC/HTS Injection Cycles

Standard Assignment for LC Injector HTC/HTS Injection Cycles	Description	Default Events	INTERFACE1 Pin #
Start JobQueue (Sync Signal)	Start a Job Queue (Job). Prerequisite is a defined "**Job**" with a method assigned. Useful for automated, unattended, timed sampling.	Immediat	
Start (Sync Signal)	Start a cycle (Input from LC or data system)	Immediat[1]	7 8(GND)
Start2 (Sync Signal)	Continue the cycle "**GC-Dual**" for the second sample	Immediat (Ignore)	
Inject (Sync Signal)	Inject READY to INJECT	Immediat (Ignore)	
Inject2 (Sync Signal)	Inject the second sample for "GC-Dual" cycle		
Injected (Out Signal)	Activated at the moment the sample has been injected	SW-Out1	3 4
Injectd2 (Out Signal)	Activated at the moment the second sample has been injected	Off	
Running (Out Signal)	Active as long as the Job Queue is being processed and the LC Injector HTC/HTS is not in an error state	Off	

[1] If used to synchronize with Sync cable, change setting to "TTL-In1"

If an item of the class “**Sync Signal**” is assigned to an output signal, such as “**TTL-In1**”, then this TTL contact must be defined as either “**Active High**” or “**Active Low**”, as expected by the HPLC system. This is defined in the Firmware class “**Events**”.



Select the corresponding item and set the mode accordingly.

The setting is provided with the schematic description of the LC Sync Cable if a dedicated cable is ordered.

PAL Software Program

PAL Loader Program

The purpose of PAL Loader Software is to create a backup file of the complete PAL System Firmware. The core software (firmware) and all PAL Firmware Objects, including all settings (Items), the local methods, and jobs are saved in one file.

The system requires PAL Loader software, “Loader” version 2.1.X or higher, and PAL Firmware 4.1.X or higher. Earlier revisions of the loader software and of the PAL firmware are not compatible. .

NOTE The new PAL Loader Software is backwards compatible. The software can be used for all PAL Firmware versions.

The name of the *.exe file has been changed intentionally to avoid conflicts when old and new versions are installed on the same computer:

- PAL Loader version 1.1.1: PALLOAD.exe

  PAL Firmware version up to and including 3.X.X
- PAL Loader version 2.1.X: Setup PALloader (.exe)

  PAL Firmware version as of 4.1.X but backwards compatible with lower versions

PAL Object Manager Software

The PAL Object Manager software is used to load a new Firmware Object to a system after a new module (hardware module) has been added to the PAL System.

The PAL Object Manager software is the software used to handle the so-called PAL Object List.

The software version 2.2.1 or higher has been developed for the PAL-xt System that, in addition to serial communication, also permits LAN communication (TCP/IP). The software is backwards compatible and can be used for all PAL Firmware versions.

The PAL Object Lists have to match the corresponding PAL Firmware version, as shown in the list below:

- PAL System Firmware Versions 2.X.X
  - PAL Object Lists Rev. K (or higher)
- PAL System Firmware Version 3.0.X
  - PAL Object Lists Rev. K (or higher) See specific Object Lists for this FW Version 3.0.X.
- PAL-xt System Firmware Version 4.1.X (or higher)
  - PAL-xt Object Lists Rev. C (or higher)

The two Object Lists for the PAL and PAL-xt Systems can be loaded with the same PAL Object Manager software. This simplifies the handling in cases where the PAL and PAL-xt Systems are in use within the same working environment.

Detailed information is available in the ‘Addendum to PAL User Manual PAL Object Manager Software’.

Establishing Communication with Computer

The System allows a serial communication protocol with a PC or LAN communication, also referred to as TCP/IP or Ethernet.

The LC Injector HTC requires a dedicated APR Control-xt board and PAL Firmware level 4.1.X or higher.

COM Port Settings

The port settings are normally defined in:

Windows\Control Panel\Device Manager\Ports.

The standard settings for the serial communication are shown in Figure 59 on page 121. It is important to understand that the application (e.g. PAL Loader Software) does actively set the communication parameters, and the baud rate is set for optimized use.

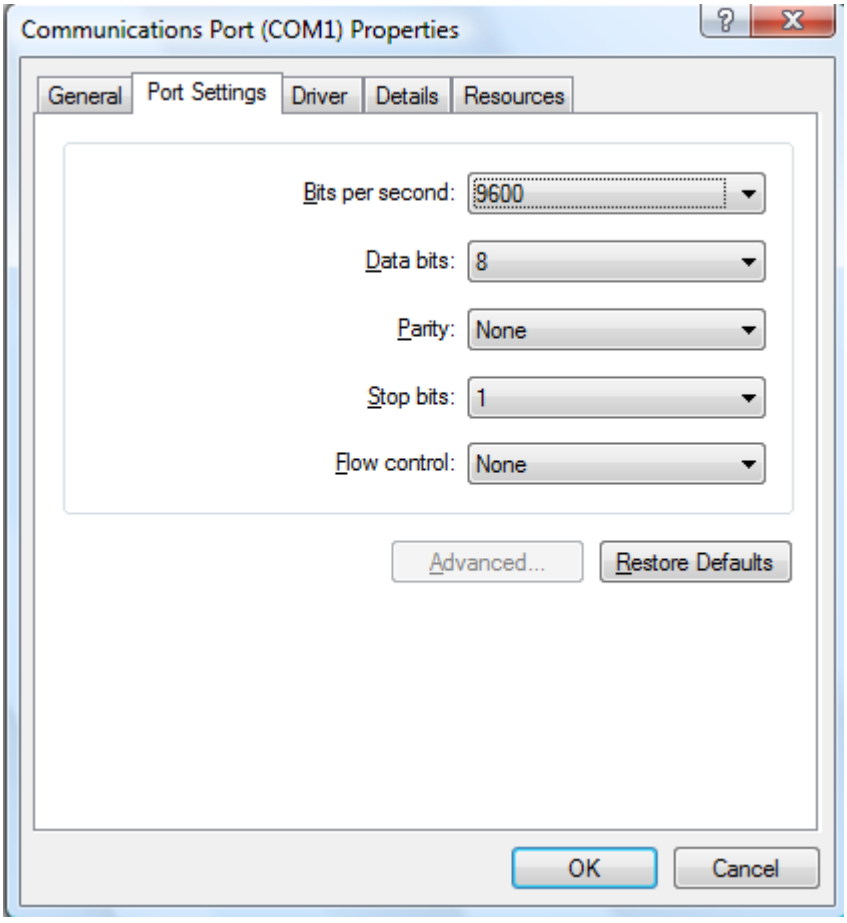


Figure 59 COM Port Settings

A quick way to check whether serial communication with the System can be established is to use the PAL Loader software.

Setting up LAN Communication

1 Select the firmware “Communication” class by the following path: Menu/F3-Setup/Communication

The system automatically searches for the MAC address when the “**Setup Communication**” window is accessed.

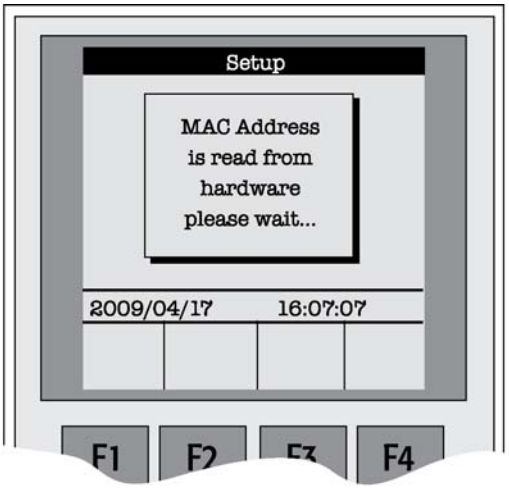


Figure 60 Reading the MAC Address from the Ethernet Communication Port

This search for and reading of the MAC address takes approximately 10 to 15 seconds. If successful, the MAC Address is stored in the system firmware and can be viewed on the terminal as follows.

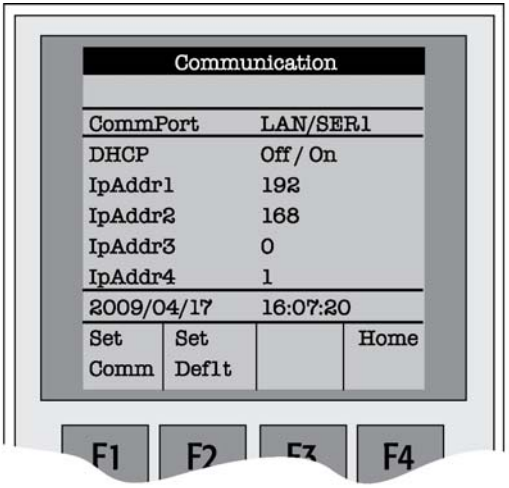


Figure 61 Communication Settings

2 Pressing function key "**F1**", "**Set Comm**" sets the selected communication mode. The same is achieved by pressing "**ESC**" or "**Home**".

Figure 62 Setting Communication Mode

Explanations for the Various Settings for the Communication Protocol

Table 32 Explanations Communication Protocol

Parameter	Explanation	Remarks
CommPort	Setup for serial (SER1) or LAN communication.	If serial communication is selected, all other parameters in PAL-xt Firmware "**Communication**" class are ignored.
DHCP	= Dynamic Host Configuration Protocol. If set to "**Off**", the IP address is provided by the user, see IPAddr1 to 4. The user can ask the network administrator for a suitable IP address. If set to "**On**", an IP address is dynamically sought throughout the provided network.	It is advisable to use a fixed IP address, set the "**DHCP**" parameter to "**Off**". In this mode the PAL-xt System always has the same fixed IP address. Troubleshooting is easier for the network administrator. See also special remarks below.
IPAddr1 to 4	Enter the IP address divided into four blocks, example IP default Address: 192.168.0.1.	The IP address is a fixed PAL-xt System address. The IP address must be provided by the network administrator.
SubNet1 to 4	SubNet mask for network configuration.	The SubNet mask must be provided by the network administrator.
TCPPort	This parameter is read only, no changes possible.	The network administrator must be informed about this setting. The port must be cleared of firewall protection to enable communication.
StdGateway 1 to 4	If Gateway is used in network, the Gateway IP address (switch, hub) has to be entered.	Gateway is used if independent networks need to communicate interactively. Communication within the network is provided by a hub or switch, defined as "**Gateway**".
MACAddr1 to6	The unique MAC Address for the PAL-xt System is read and entered into this PAL-xt communication protocol. This is a read only parameter field.	The MAC address is provided by the Ethernet communication hardware. The address is also visible beside the readout from the terminal on the label affixed to the Ethernet module on the PCB "**APR module**".

Special Remarks to the above listed Parameters

DHCP:

In current Firmware level 4.1.X, certain limitations accompany the “**DHCP**” communication protocol. If “**DHCP**” mode is active, a dynamic search for the IP address is activated. If a free IP address is found, the communication from the PAL-xt System to the network becomes established. The new IP address (found through “**DHCP**” search) is not entered into the PAL-xt Communication protocol as seen from the local terminal. The parameters “**IPAddr1 to 4**” are not updated.

How to overcome this situation is described below with the PAL Loader software as example but analogously applies the described path to other application as well.

The Setup for the PAL Loader software requires entering the IP address or hostname. The dynamically provided IP address cannot be displayed on the local terminal. To overcome this difficulty, the following setup procedure can be used:

Preparations PAL-xt setting in “**Communication**” class: **DHCP = On**.

1 Open the “Setup” window and enter the following name in the “**Hostname or IP Address**” field: CXXXXXX. The “**Xs**” must be replaced by the last six characters of the MAC address, which is known from the PAL-xt System as parameters “**MacAddr4**”, “**MacAddr5**”, and “**MacAddr6**”.

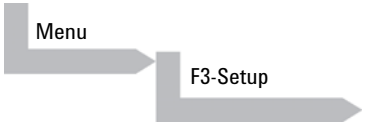
Special Functions

This section describes special functions which can be activated by the "**PAL Extended User Mode**".

The purpose of having two software access levels is to display discrete items and sections of the firmware at each level. Settings which need to be done rarely, perhaps at the time of installation of the System, are hidden at level 1(User Level) and revealed at level 2 (Extended User Level). This also protects the System in group use. Nevertheless, the important points should be known by the user and are described below.

How to Access "Extended User Mode"

Extended User Mode can be accessed by the following path:



The above path as shown is an abbreviation for the following steps:

1. **Menu**/ scroll the cursor to **Setup**/
2. Press "**F3**" /
3. Activate "**Setup**" by pressing the center of the selector knob.

In this User Manual or in technical notes, only the following will be referred to:

Menu > F3-Setup.

Section "F3-Setup"

The path to access this section is shown in Figure 63 on page 127. This "**Setup**" section at the Extended User level provides various options to access other classes which are not visible at the User level. Some classes are known but may show some new items. Details are described in Table on page 128.

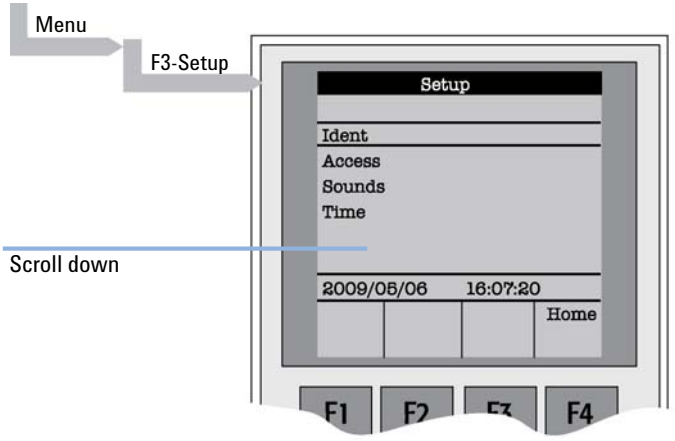


Figure 63 Selecting "**Setup**" in Extended User Mode

Object	Description
Ident	The "Identification" of the system provides the opportunity to assign Users, site, and system names. The serial number of the System can be entered at this level. Remark: After loading a fresh Firmware Backup file, the SNo. is displayed as "XXXXXX".
Access	Access can be selectively opened or limited to different users for the "**Job**", "**Method**" or "**Setup**".
Sounds	The "Beep" sound can be adjusted to specific needs. For safety reasons it is advisable to keep the default settings, especially the "**Warn Move**", turned ON to signal the start of the System to the user.
Time	The internal clock can be set. Remark: If the clock is fast, reset all items, from "**Year**" to "**Seconds**" and use the mandatory "**F1**" "**Set Time**".
Communication[1]	The communication mode, serial or LAN, can be selected and conditions are defined.
System[2]	Basic parameters can be predefined for specific needs. Details see "System" on page 129.
Service[2]	Basic service tests can be activated. Details see "Service" on page 132.
Objects	Accessing the Firmware class "**Objects**" in the Extended User mode provides more detailed possibilities for object items. At this level an Object can be copied or deleted by using the specific function keys. This provides quick access to add e.g. a second or third injector.

1 Available beginning with PAL Firmware Version 4.1.X.

2 Explained in detail below.

System

The following "**System**" Items can be changed by selecting the particular item.

Beginning with Firmware version 4.1.X, an intermediate step was added. After selecting "**System**", the new "**Settings**" level follows. Activating "**Settings**" opens up the described Items below.

Item	Description
Syr Detect	The syringe detection can be turned off in cases of sensor failure or if a special syringe with unknown ID is installed. Turning OFF the syringe identification sensor automatically displays a dialog box enabling the selection of syringe identification manually.
Start Ref	This item provides the choice of referencing all axes at the start of the system or suppressing the referencing. It is advisable to use this function actively to detect a possible loss of steps.
Stop XY Error	This function allows turning off the automatic recovery of the system after detection of a collision (loss of steps of a stepper motor). **Stop XY Error = ON**: Automated recovery turned OFF **Stop XY Error = OFF**: Automated recovery turned ON In Firmware version 2.3.X the new feature that recovers automatically after detecting a collision has been implemented. Firmware version 2.6.2 provides the possibility of disabling automated recovery. If recovery is turned OFF, the system always checks its position before moving to the injection port for injection. This can lead to an extra move, crossing over the X-,Y-axes sensors – if the injection port is at the opposite end of the X-axis relative to the sample location, (zero X-axis position in-between sample location and injection port). For critical application fields, e.g. clinical analyses, it is advisable to turn off the automated recovery; **Stop XY Error= ON**.
PlgPathCheck[1]	The plunger travel path is monitored to ensure that the plunger tip reaches the syringe zero point in all cases – compared to original plunger referencing and defining the syringe zero point. If an error is detected, the System stops operation. This feature is useful if sample solutions with particles need to be handled, or if the syringe type is not adequate for the solution composition, or if the syringe plunger is blocked or even bent, such an error will be recognized and the system will come to an emergency stop.
PlgChnge Pos	Changing a syringe using the Menu function "**Chnge Syr**" moves the plunger up to the position specified by this item. For normal use, accept the default values.

Item	Description
Init Syr at	The syringe plunger can be initialized (referenced) at "**Home**" or "**Waste**" position. If samples with a high danger of toxicity are treated, it is advisable to use the "**Waste**" position for referencing.
Inj Signal	An Autosampler is usually the master in a total chromatographic system. The other components send a "**Ready**" signal to enable a start of the Autosampler. At the moment of injection a Start signal is sent from the System to the other component(-s) of the entire system. With this "**Inj Signal**" item the status at which the signal is sent out can be defined. The syringe is filled with the defined sample volume. **PlgUp** = Start signal at the moment the plunger starts moving down for injection. **PlgDown** = Start signal at the moment the plunger reached zero point from syringe (recommended for large volume injection, but not in combination with Agilent Chemstation). **ValveSw** = Start signal sent at the moment the injection valve is switched, HPLC technique. **PreInj** = Start signal sent at a time before injection, as with a sample preparation device started before a GC or LC system. Minus time relative to chromatographic start time.
Vial PrePress	This item can be activated to avoid a vacuum if a relatively large amount of sample is taken out of a small volume vial. If e.g. a volume of 50 µL is specified for injection, the PAL system first takes 50 µL ambient air and "injects" this volume into the sealed vial, causing overpressure in the vial. After this step, the sample volume can be aspirated without forming a vacuum. This functionality is only valid for liquid sampling, not for the Headspace technique. Starting with Firmware version 4.1.X, this item is also active in combination with Cycle Composer.
LC-Inj	Standard cycle for HPLC technique. Details on method parameters see "Injection Cycles" on page 196" and "Software Flow Chart" on page 246.
LC-Cut	Cycle for column switching with two valves, HPLC technique. Both valve drives have to be controlled through "AUX" interfaces. Details on method parameters see "Software Flow Chart" on page 246.
GC-Inj	Standard Cycle for GC technique.
GC-InS	Standard cycle covering GC Sandwich technique.

Item	Description
GC-Dual	Cycle for GC technique which allows injecting two samples in two different injectors. The start signal is sent after injection of both sample solutions.
HS-Inj	Standard cyle for GC headspace technique.
SPME	Standard cycle for GC SPME technique, Solid Phase Micro Extraction, Fiber technique.
TTS Sotax	The two cycles are dedicated cycles for “dissolution applications”. TTS cycle is used for transdermal patches and the Sotax cycle is used for tablet dissolution.

[1] Available beginning with Firmware version 4.1.X.

NOTE Standard injection cycles are selectable and activated directly from the System. Any deviation from a standard cycle, a Cycle Composer Macro, or Cycle Editor (ICC-CE) Cycle must be programmed for customized requirements.

NOTE In Firmware versions < 4.0 the standard cycles are grouped in Item “**Cycles**”.

Service

"**Service**" items may be changed by selecting the particular item (see Table on page 132).

Item	Description
Check Motors	Every stepper motor can be moved separately. This function is useful to check out a specific motor or to perform an endurance test after a repair. The Start and Stop positions of the test path can be selected. The motor Current, Speed, and Acceleration are selectable (accept default value to be sure). A Pause time can be specified. A Cycle Limit and Actual Cycle Counter are useful tools for endurance tests. **Phase A**: This item is used to verify the functionality of the stepper motor. The current can be manually increased for tests. Used for troubleshooting only. **Phase B**: This item is used to verify the functionality of the stepper motor. The current can be manually increased for tests. Used for troubleshooting only. **MicroStep Inx**: The motor can be moved within a range of up to four full steps by applying the assigned current. **NOTE** These items are typically used for service. If applied, do not leave the test settings for longer than a few minutes. If current setting is in a critical range the motor can be damaged.

Item	Description
Test Head	This tests the functionality of the Injection Unit ("**Head**"). Two tests can be activated by the function keys: "**F1**" **Check Plg**: The stepper motor of the plunger drive is tested. Sensor check. "**F2**" **Check Z**: The vial detection sensor and "**Needle Guide Blocking**" function is tested. These tests are only required if any erratic behavior of the System is observed. In such cases contact a representative of Agilent Technologies.
Test Switches	**X-Limit** = Actual status of X-axis sensor. Move the X-Y-Carriage manually across the limit switch to verify activation of the sensor. **Y-Limit** = Actual status of X-axis sensor. Move the Y-axis manually to the Y-zero point (at X-axis), crossing over the limit switch to verify activation of the sensor. **Z-Limit** = Actual status of Z-axis sensor. Move the syringe slider assembly manually up to the zero Z-axis point, crossing over the limit switch to verify activation of the sensor. **Aux1 Limit** = Actual status of Aux1 limit sensor **Aux2 Limit** = Actual status of Aux2 limit sensor **Plg –Pos** = Actual status of Plg –Pos **Needle Guide** = Actual status of needle guide sensor

Section "F3-Setup" / Objects

The same as is described above applies for the section "**Objects**" opened in Extended User Mode. New classes are visualized and known classes may contain new items accessible only on the level of the Extended User Mode.

To open this level, use the following path:

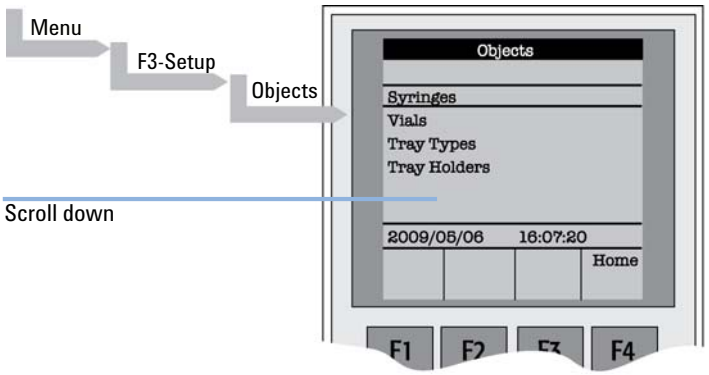


Figure 64 Selecting "Objects" in Extended User Mode

Object	Description
Syringes	At this level all loaded syringes are selectable. At the "**Utilities**" user level only the active syringe is visible.
Vials	The Firmware Objects accessed on the Extended User Mode have more specific items selectable than at the User level. Tolerances or ranges such as, e.g. "**Maximum Penetration**" can be defined and limited for the user such that this value cannot be exceeded. In the Object "**Tray Types**", details for the various Items are described more closely.
Tray Types	The object class "**Tray Type**" is only accessible via the Extended User Level. All loaded Tray Types are visible and selectable. Details, see "Tray Type" on page 138.
Tray Holders	The Firmware Objects accessed on the Extended User Level have more specific items selectable than at the User level. Depending on the type of Tray Holder, e.g. an Access and Restore Path or a Heater and Agitator (motor) is assigned. Typical example, the Tray Holder "**Agitator**".

Object	Description
Trays	All loaded Trays are visible and accessible. A Tray must be assigned to a Tray Holder. A "**TrayTypeGroup**" identification checks if the specified Tray Type is allowed in this combination. The offsets X-,Y-, Z and the inclination corrections for a row or column are explained in the "**Utilities Functions**" section. The "**Path Offset X-,Y-, Z**" is used only if a "**Path**" is assigned to the specified Tray Holder, typically a Stack or Agitator uses a Path.
Wash Stations	The Wash Station and its positions "**Wash1**" and "**Wash2**" are visible and selectable. Details , see "Wash Stations" on page 144.
Injectors	The Firmware Objects accessed on the Extended User Mode have more specific items selectable than at the User Level. Tolerances or ranges such as "**Maximum Penetration**" can be defined and limited for the user so that this value cannot be exceeded. In the "**Tray Types**" Object, details for the various Items are described. Furthermore, in this Firmware Object class a "**Valve**" and/or a "**Heater**" can be assigned. Note that the Wash Station Positions "**Waste**" and "**Waste2**" are listed in this "**Injectors**" class.
Valves	The Valves and Valve Drives connected and controlled by an "**AUX**" interface are listed in this Object class. For more details, see the Valves and Valve Drives Addendum. Note that the valves controlled by serial communication (Serial Valves or Multiposition Valves) are in the Firmware Object class "**Serial Valves**".
Dilutors	The Firmware Objects accessed in the Extended User Mode have more specific items selectable than at the User level. Tolerances or ranges such as "**Maximum Fill Speed**" can be defined and limited for the user so that this value cannot be exceeded. Items such as "**Prime Volume**", "**Waste to**", "**Motor Drives**", or "**Scale Length**" are specific items that remain hidden at the User level.
Positions	The various positions used with the System are selectable at this level. Positions such as "**Home**", "**Change Syringe**", or a Path Point, reference the Paths for a specified Tray Holder directly, such as "**Agitator**" or "**Stack**". Selecting an item, e.g. "**Home**" will provide "**F1**", "**Check Pos**" on the screen. Activating this function provides the possibility of verifying X-, Y-, Z-axes values for this particular position.

Object	Description
Paths	A Path defines specific movements for the Injection Unit, which represent a repetitive task assigned to a Tray Holder. Typical examples are opening and closing a drawer from a Stack or opening the lid of an Agitator. The Path is composed of several steps (points) which are added up in a sequence. Each individual step (point) can be positioned by teaching X-, Y-, Z-axes. In most cases the return path is in the reverse order of the starting path. Example "**AgiOpnL**" and "**AgiClsL**". If one setting of a point is changed, this change will be valid for the return path as well.
Sync Signals	The various synchronization signals are described in "Synchronization and Output Signals" on page 115. At this level no other functionality, as shown at the user level, is provided.
Out Signals	The various Out-Signals are described in "Synchronization and Output Signals" on page 115. At this level no other functionality, as shown at the user level, is provided.
Events	The various synchronization signals are described in "Synchronization and Output Signals" on page 115. At this level no other functionality, as shown at the user level, is provided. Three events are newly available at this extended user level: **Pwr-Out1**, **Pwr-Out2** and **FlushVlv**. The main purpose of these three power-out signals is to activate a solenoid, such as for the Wash Station or the Gas Flush Valve. At this level the functionality of the signal can be tested.
Serial Valves	Serial valves, or "Multiposition Valve Drives" are powered and controlled by the serial (RS232) control and not through the "AUX" interface. With firmware level 4.1.X a "**Mode**" is now selectable. The mode refers to the setting of the Valve Drive, whether the current for the motor is set to "**High Speed**" or "**High Torque**". For more details, see Addendum to PAL User Manual "Valve and Valve Drive".
Out Exp Box	The "Out Expansion Box" provides 8 TTL contacts and 8 relay contacts (24 V contact closure). The optional module (box) is connected and controlled by "Interface 2". For more details, see Out Expansion Box Addendum to PAL User Manual.
RS232	This Object allows selecting the serial ports for the specific needs. Choices are: **Remote**, **Terminal**, **Printer**, **Barcode**, **ExtDev**, **VICI-Vlv** (for serial valve drives), or **None**.

Object	Description
Motors[1]	In the class "**Motors**" the Objects "**Motor-Y**" and "**Motor-Z**" are accessible to enable the user to select specific motor parameters in case a specific application requires an adjustment. **Motor-Y Strategy** Three different settings are selectable for the Y-Motor movement strategy: • **Rectang** = normal movement as used for system. (In firmware version < 4.1.X. this mode was called "**Auto**".) • **XY-Simul** = X,-Y-axes move simultaneously, which allows movement in a diagonal direction. (At firmware version < 4.1.X this mode was called "**Rectang**", but it was not active at lower versions.) • **Retract** = Y-axis moves from any point first to zero Y (back to the X-axis) before the X-movement is started. This mode allows bypassing an object (such as a large GC detector) which may be in the travel path if the default mode "**Rectang**" is used. • **Path Speed** = Y-axis motor speed in conjunction with a "**Path**", example: Opening and closing a drawer of a Stack. In certain situations it is necessary to slow down the speed to open and close a drawer. Typical application example is fraction collection, collecting directly into a well plate. If the liquid level is high, the liquid can wash over into the next row if drawer movement is too fast. **NOTE** At Firmware level 4.1.X these items of the Y motor have been made available at the Extended User Level. **Motor-Z** For the 'Motor-Z', following speeds are selectable: • **Penetr Speed** = This is the speed used to penetrate the syringe needle into any object, with the exception of the injector. Example: Vial penetration. • **Inj Penet Spd** = Injector Penetration Speed. With PAL Firmware version 2.X and 3.0 the injector penetration speed is identical to the injector retraction speed. • **Inj Retr Spd** = Injector Retract Speed. With PAL Firmware version 4.1.X, the injector speed was separated into a penetration and a retraction speed. This allows safe penetration of a vial by any type and diameter of syringe needle without bending through the septum of the GC injector. The needle retraction speed can be increased to speed up the entire injection process. This new feature enables the user to avoid uncontrolled evaporation of any remaining sample solution in the needle. The visibility of the Motor-Z parameters at the Extended User Level was introduced with PAL Firmware version 4.1.3.

[1] Available beginning at Firmware Level 4.1.X

Tray Type

The following object “**Tray Type**” Items may be changed by selecting the particular item. First select the desired Tray Type, such as “**VT32-20**”.

NOTE

The definition of Row and Column is given in “Definition of a Tray Row and Column” on page 94.

By reversing the first six entries, the order of injection can be changed.

Example: Tray Type “**VT32-20**” has a “**Row Length Y**” and a “**Col Length X**” defined. Reversing to “**Row Length X**” and “**Col Length Y**” would start with sampling from left to right, as used e.g. with Tray Type “**DW96**”.

The values of the items “**Spl Per Row**” and “**Spl Per Col**” must also be reversed.

Item	Description
Row Length X	Defines the Row length in the X-direction from the center of the first to the center of the last vial position, measured in mm.
Row Length Y	Defines the Row length in the Y-direction from the center of the first to the center of the last vial position, measured in mm.
Col Length X	Defines the Column length in the X-direction from the center of the first to the center of the last vial position, measured in mm.
Col Length Y	Defines the Column length in the Y-direction from the center of the first to the center of the last vial position, measured in mm.
Spl Per Row	Number of samples per Row.
Spl Per Col	Number of samples per Column

Pattern[1]	The pattern of the Row or Column arrangement has to be defined.
	• **Regular** = square arrangement (standard)
	• **Staggrd+** = Rows are arranged in staggered pattern.
	  ◦ Offset of second Row is shifted by +50 %5 of hole pattern.
	• **Staggrd-** = Rows are arranged in staggered pattern.
	  ◦ Offset of second Row is shifted by -50 % of hole pattern.
	See Graphics below.

TrayTypeGroup	Software protection so that not every Tray Type can be placed on any Tray Holder. Example: VT32-20 cannot be fit into a Stack.
Plate Thickn	Thickness of Tray plate bottom (not the total height of the Tray). Measured in mm.

Item	Description
Vial Height	Total height of the vial including cap to seal if applicable. Measured in mm. **NOTE** The sum of the plate thickness and vial height adds up to the total height This is the point where the Injection Unit expects an object.
Vial Trnsprt[2]	The mode to transport a vial is defined by: • **None** = No transport required. Example liquid sampling. • **Magnet** = Magnetic Transport. Requires magnetic vial caps. The Injection Unit moves to the side to slide the vial off when moved away. • **Needle** = Device is transported with the syringe needle inserted. Example: special vials where a magnetic cap cannot be crimped, e.g. **Vacutainer**.
ZSlideOffRetr[1]	If magnetic transport is selected, the distance can be defined to move the syringe slider up (Z-direction), lifting up the vial by magnetic force. Measured in mm.
YSlideOffDist[1]	If magnetic transport is selected, the distance can be defined to move the Injection Unit to the side, Y-direction, to slide the vial off. Measured in mm.
Barcode	If a Barcode Reader is installed, the mode has to be set for **AutoFix**. **None** = No Barcode Reader option requested for this Tray Type.
Z Tolerance	A tolerance window to give a plus/minus range (expressed in mm) where the Injection Unit must expect an object. If the value of this item is > 0, this is a relative detection mode and the sensor from the Injection Unit needle guide is active. If the value of this item is set to 0 the sensor of the needle guide is turned off and the syringe slider moves to an absolute value, Z-axis position as specified in the object.
Z Retract	The distance to lift up the syringe slider before the Y-, Z-axes assembly moves across the unit can be specified, expressed in mm.
Max Penetr	The maximum allowed Needle Penetration can be defined as a safety so that the user cannot destroy the needle tip by going too far down. The Tray Type is prepared for a specific vial size; this value is therefore unique to this combination. The maximum needle penetration must not exceed the length of the syringe needle, considering the loss due to needle guide, etc.. Typical needle length is 51 mm, maximum penetration must not exceed 48 mm.
Needle Penetr	This item is identical to that used on the User Level or, if available, as a method parameter of a local cycle. The value defined on these levels is mirrored to the Extended User level.

Item	Description
Spl1 Offset X	The X offset is defined as the distance from the outer edge of the Tray in the X-direction to the center of the vial position 1. Measured in mm.
Spl1 Offset Y	The Y offset is defined as the distance from the outer edge of the Tray in Y-direction to the center of the vial position 1. Measured in mm.
Spl1 Offset Z	The Z offset can be selected for special cases where, e.g. the syringe needle has to reach a deeper point before penetration into a vial. A Z offset value = 0 considers the Tray surface or vial top as the reference point. Measured in mm.

[1] Available beginning with Firmware version 4.1.X

[2] Available beginning with Firmware Level 2.5.X

NOTE The dimensions used for Row/Column Length or X-, Y-, Z-Offsets for Sample Number 1, are specific for a particular Tray Type. If a general adjustment for a Tray from a specific vendor – e.g. for a Deepwell Plate – has to be made, this should be done in the "**Tray Type**" class of Object. For fine tuning, the Offset items from the class "**Trays**" can be used.

Explanation for various Patterns in Tray Type:

1 Pattern Type "**Regular**".

The sample or well positions are equal distributed.

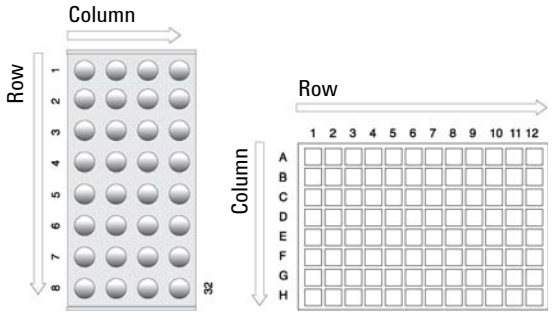


Figure 65 Pattern Tray Type "**Regular**"

2 Pattern Type "**Staggrd+**".

The sample or well positions are arranged in a staggered pattern. Offset of second Row is shifted by +50 % of hole pattern.

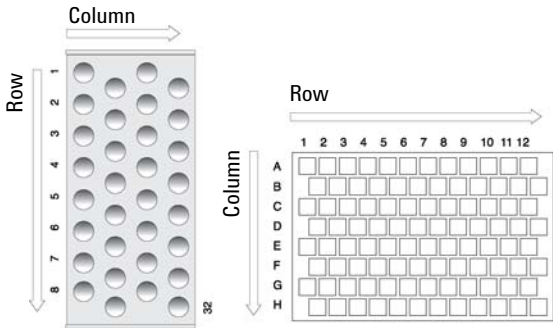


Figure 66 Pattern Tray Type "**Staggered+**"

3 Pattern Type "**Staggrd-**".

The sample or well positions are arranged in a staggered pattern. Offset of second Row is shifted by -50 % of hole pattern.

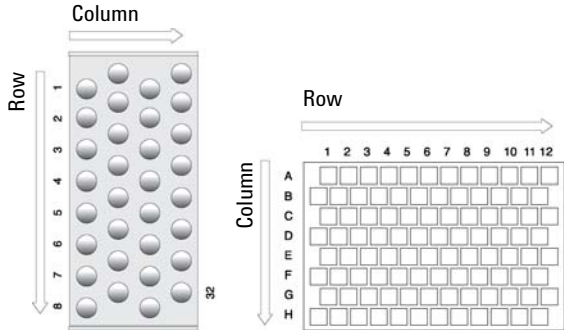


Figure 67 Pattern Tray Type "**Staggered-**"

Trays

The following object "**Tray**" Items can be changed by selecting the particular item. First select the desired Tray, such as "**Tray1**".

Item	Description
Tray Holder	The Tray Holder to which the Tray is assigned is specified.
TrayTypeGroup	All Tray Type Groups which are allowed for this particular combination of Tray and Tray Holder are displayed. The **TrayTypeGroup**for the Tray Type itself is specified for each Tray Type.
Tray Type	The Tray Type to be used is linked to the Tray. This item is usually set at User level in **Utilities > Trays**. See above.
Offset X	The X offset is defined as the distance from the center of the teaching position from the Tray Holder to the edge of the Tray in X-direction. Measured in mm. This item is available at User level in section **Utilities > Tray**and can be used for fine tuning a Tray installed in a Tray Holder. Important for well-plates 384 installed in a Stack. **NOTE** Position#1 is the only correction point.
Offset Y	The Y offset is defined as the distance from the center of the teaching position from the Tray Holder to the edge of the Tray in Y-direction. Measured in mm. This item is available at User level in section Utilities/Tray and can be used for fine tuning a Tray installed in a Tray Holder. Important for well-plates 384 installed in a Stack. **NOTE** Position#1 is the only correction point.

Item	Description
Offset Z	The Z offset is defined as the distance from the plate surface of the Tray Holder to the teaching point, black needle guide flush underneath plate of Tray Holder (which corresponds to plate thickness of Tray Holder). Measured in mm. This item is available at User level in section Utilities/Tray and can be used for fine tuning a Tray installed in a Tray Holder. Important for well-plates 384 installed in a Stack. **NOTE** Position#1 is the only correction point.
Path Offset X	The Path Offset X can be specific for a Tray, adjustment in X-direction. Expressed in mm. **NOTE** This item is only valid if a Path is assigned to the Tray Holder as specified in this combination.
Path Offset Y	The Path Offset Y can be specific for a Tray, adjustment in Y-direction. Expressed in mm. **NOTE** This item is only valid if a Path is assigned to the Tray Holder as specified in this combination.
Path Offset Z	The Path Offset Z can be specific for a Tray, adjustment in Z-direction. Expressed in mm. **NOTE** This item is only valid if a Path is assigned to the Tray Holder as specified in this combination.
dxRow[1]	Correction for inclination of a Tray in X-row direction. For details, see "Correction for Sample Position" on page 95.
dYRow[1]	Correction for inclination of a Tray in Y-row direction. For details, see "Correction for Sample Position" on page 95.
dzRow[1]	Correction for inclination of a Tray in Z-row direction. For details, see "Correction for Sample Position" on page 95.
dxCol[1]	Correction for inclination of a Tray in X-column direction. For details, see "Correction for Sample Position" on page 95.

Item	Description
dyCol[1]	Correction for inclination of a Tray in Y-column direction. For details, see "Correction for Sample Position" on page 95.
dzCol[1]	Correction for inclination of a Tray in Z-column direction. For details, see "Correction for Sample Position" on page 95.

[1] Available beginning with Firmware Version 4.1.X

Wash Stations

The following object "**Wash Station**" items may be changed by selecting the particular item. First select the desired Wash Station, such as "**Wash1**".

Item	Description
Position X	X-axis position for Wash Station.
Position Y	Y-axis position for Wash Station.
Position Z	Z-axis position for Wash Station.
Z Tolerance	A tolerance window for a plus/minus range (expressed in mm) where the Injection Unit has to expect an object. • If the value of this item is > 0, this is a relative detection mode and the sensor from the Injection Unit needle guide is active. • If the value of this item is set to "0" the sensor of the needle guide is turned off and the syringe slider moves to an absolute value, Z-axis position as specified in the object.
Z Retract	The distance to lift up the syringe slider before the Y-, Z-axes assembly moves across the unit can be specified, expressed in mm.
Max Penetrat	The maximum allowed Needle Penetration can be defined as a safety so that the user cannot destroy the needle tip by going too far down. The maximum needle penetration must not exceed the length of the syringe needle, considering the loss due to needle guide, etc.. Typical needle length is 51 mm, maximum penetration must not exceed 48 mm.
Needle Penetr	This item is identical to that at the User level. The value defined at this level is mirrored to the Extended User Level.

Item	Description
Type	The specific mode for the Wash station in use has to be defined: • **Standard** = Standard Wash Station. Syringe is filled with wash solvent and expelled into Waste. • **Pulse** = A pulsed signal is sent to activate the device. Used for the "**Fast Wash Station**" and "**Active Wash Station**". See Type `**Active**´ for details. • **Flow** = Continuous flow of liquid by gravity. No electrical contact required for a solenoid. • **DLW** = Dynamic Load & Wash. Item used to activate specific solenoid for DLW Wash Station. • **Active** = Used for Active Wash Station in conjunction with the Wash Station Print with PNo. 'APR WASHPUMP B.A.' (Revision B). Simplified print, activation of pumps is controlled by PAL Firmware. Requires Object Lists 'PAL WashActive_Rev. B'.
Flow Control	The power source to activate a Wash Station is specified. For the Fast, Active Wash Station and DLW the Event "**Power-Out1**" and "**Power-Out2**" are used.
Ndl Cln Path	A Path can be assigned to the Wash Station.
Waste to	The Waste position is assigned to the Wash Station.
Clean Volume	A percentage of the syringe maximum volume is defined for the cleaning step.
Clean Count	The counter monitors the number of wash cycles. The setting in the method has the same functionality and the value is mirrored to the Extended User Level.
Max Fill Spd	The fill speed for the syringe is defined with this item. If the maximum value of the Fill Speed (10.0 ml/s) is selected, the system takes the injection syringe Fill Speed as defined in the method.
Max Eject Spd	The Eject Speed is the speed used to eject the wash solvent during the wash cycle. This speed is usually higher than the Fill Speed. If the maximum value of the Eject Speed (10.0 ml/s) is selected, the system takes the injection syringe Eject Speed as defined in the object class "**Syringe**".
Rinse Time	Rinse is the time to rinse the wash port with wash solvent after the wash cycle of the syringe has been finished. In the case of a Fast/Active Wash Station, the syringe needle is pulled out of the wash port. The wash solvent flows without restriction. As a result, higher solvent consumption has to be taken into consideration.

Troubleshooting

NOTE The autosampler represents always one of several components in a chromatography system.

The following Troubleshooting Guide is limited to the autosampler only.

No or very low detector signal is observed.

Probable cause	Suggested actions
1 Clogged syringe.	Remove syringe and aspirate/dispense liquid manually. Clean syringe.
2 Bent needle.	• Check X-,Y-, Z-Axes positions. • Check vial or well-plate septum.
3 No sample liquid is injected.	Check and/or adjust Needle penetration into sample vial (see "Utility Functions" on page 27).
4 Sample volume too low.	Increase sample volume.
5 The valve needle guide and / or needle seal are not properly installed.	Check valve needle guide and seal (see "Valve Needle Guide" on page 112).
6 The valve ports are not plumbed correctly to the pump and/or detection system.	Check plumbing connections (see "Injection Valve Flow Path" on page 106).
7 Wrong valve type specified.	Check valve type by selecting the path: **Menu > setup > Objects > Injectors > LC Vlv1 > valve**.
8 Rotor orientation	Check the rotor orientation. (This possibility is valid for Rheodyne valves only.)

Mobile phase and/or sample flows out of the wrong ports on LC injection valve.

Probable cause	Suggested actions
1 The valve rotor is reversed (180 ° out of alignment).	Remove the rotor and reinstall in the correct position. See "Injection Valve / Valve Rotor" on page 173).

Sample is backing up on the valve needle guide.

Probable cause	Suggested actions
1 High restriction in valve flow path.	Check the restriction of sample loop, valve waste tube or clogged connection (ferrules or tubing distorted).
2 Valve needle seal leaks.	• Check needle seal type. • Change valve needle seal (see "Valve Needle Guide" on page 112).
3 The needle penetration depth for the injection valve is not set correctly.	Adjust the Injection Valve Needle Penetration .
4 The syringe Plunger speed is too high resulting in excessive pressure in inlet.	Reduce Inject Speed in method.

Syringe does not fill properly.

Probable cause	Suggested actions
1 DLW Actuator/Solenoid not functioning.	PAL Firmware level < 4.0 or PAL Firmware Object for '**DLW Option**' not loaded onto the system.
2 Solvent path blocked.	Prime the system or inspect it to identify the reason for clogging.
3 Air bubbles trapped in the system, caused by loose connections or tubing not cut square.	Ensure tight tubing connections at all points; verify that all tube ends are cut properly.
4 Syringe plunger tip worn out.	Replace syringe plunger. Check if glass barrel is scratched (damaged). If in doubt, replace the entire syringe at once.
5 DLW-2 Actuator Control PCB defect.	If possible, verify the DLW Actuator Holder Assy. on another System. If there is a definite defect, replace the PCB.
6 DLW-2 Holding Loop.	Check the DLW-2 Holding Loop. Is the solvent flowing freely in the loop?
7 Injection Needle (Holding Loop).	Check the front section of the holding loop. - Is the needle entry free of debris, not clogged?

Sample peaks or responses are not reproducible.

Probable cause	Suggested actions
1 Dirty syringe.	Use the '**Priming**' utilities as described in Section 'Operation'.
2 Syringe pressure differences.	Increase Pullup Delay value.

Probable cause	Suggested actions
3 Vacuum created in sample vial.	Reduce sample volume in sample vial. Use setting under **F3-Setup > System > PrePressureVial**.
4 Method Parameters	Use the macro default settings and observe the following crucial parameters first: **Fill speed**, **Pullup delay**, **Injection Speed**, **Post Inj Delay**. Do NOT use the standard injection cycle 'LC-Inj'. Please note that this cycle cannot be used for the DLW operation. However the values of these parameters can be used as a good starting point.

Peak distortion or tailing.

Peak distortion or tailing

Probable cause	Suggested actions
1 Method Parameters.	See recommended actions above.
2 Solvent for analytical solution not suitable.	Change solvent composition to a lower polarity (in case of reversed phase chromatography).
3 Tubing internal diameter.	Check tubing internal diameters for connection tubings and injection loop. See recommendations as given with poster 'Tips&Hints for HPLC Technique' (CTC Anayltics).

Probable cause	Suggested actions
4 Bad connections.	Connection tubing's not square cut or wrong type of nuts or ferrules installed. See recommendations as given with poster 'Tips&Hints for HPLC Technique' (CTC Analytics).
5 HPLC related problems.	Any parameter like flow rate, composition of mobile phase or wash solvent, column selection (diameter, pore size, etc.) can contribute to BAD PEAK SHAPE. Check troubleshooting guide from the HPLC manufacturer or any other source.

Excessive carryover between samples

Probable cause	Suggested actions
1 Loose, unstable or wrong connection.	Check all connections within the DLW-2 System, such as Holding Loop, injection loop connections, or valve plumbing connections etc.
2 Inappropriate wash solvent(s).	Use appropriate wash solvent(s).
3 Waste tubing ID at injection valve is too small, causing waste liquid to be pulled back, by capillary action, into rotor groove.	Replace the waste tubing with larger ID tubing.
4 Air gaps not formed. Liquids of different type diffusing into each other.	• Verify the DLW method parameter for 'Airgap Volume'. • Prime the entire system.
5 ID of solvent tube from injection valve to DLW Wash station is too small.	ID of 0.8 mm (1/32") is required as a minimum internal diameter to avoid any restriction.
6 Dirty needle, Holding Loop and/or valve injection port.	Use the 'Priming' utilities as described in Section *Operation*
7 Damaged or grooved valve rotor.	Replace valve rotor, see "Injection Valve / Valve Rotor" on page 173.

Probable cause	Suggested actions
8 Valve needle seal leaks.	Change valve needle seal, see "Valve Needle Guide" on page 112).
9 Inappropriate wash solvent composition.	Use appropriate wash solvent. Observe also the order of use for biological samples. The first wash is always an aqueous solution followed by organic solvents.
10 DLW Actuator/Solenoid questionable, defect.	Verify the functionality of the DLW Actuator. Is solvent flowing when active? Use command "**Utilities/Wash Station**" for tests.
11 Solvent Frits in wash solvent reservoir blocked.	• Clean the frits in a ultrasonic bath with an appropriate solvent. • If flow is not ensured after cleaning, replace frits. • Replace wash solvent and clean reservoir bottle and tubing carefully.
12 Inserts of Wash station contaminated or clogged.	Clean all parts of the DLW Wash Station by using an appropriate solvent (according to the application) and if possible an ultrasonic bath.

Unexplained collisions of the Injection Unit.

Probable cause	Suggested actions
1 Object(s) not defined correctly.	Define Object(s) correctly. (See "Defining Object Reference Positions" on page 88).

“Object Not Detected” (Object e.g. Tray1, LCVlv1, Stk1- 01)

Probable cause	Suggested actions
1 Incorrect Z value for the affected Object (e.g. LCVlv1, THldr1 or CStk1-01).	Redefine Z value for the affected Object. (See “Defining Object Reference Positions” on page 88).
2 Missing sample vial, Deepwell- or Microtiterplate.	Check position and insert, if necessary, vial, deepwell- or microtiter plate.

“Object Collision before Z Tolerance” (“Object” e.g. Tray1, LCVlv1, Stk1-01)

Probable cause	Suggested actions
1 Incorrect Z value for the affected Object.	Redefine Z value for the affected object. (See “Defining Object Reference Positions” on page 88).

“Invalid Motor Status Motor AUX1”. (AUX2)

Probable cause	Suggested actions
1 Injection valve not connected.	Switch OFF LC Injector HTC/HTS and check connection between LC Injector HTC/HTS and Valve Drive.
2 Defective LCVlv1 Connection cable.	Change connection cable.
3 Control Board defective.	Replace Control-HTC or Control-xt Board.

Special Considerations for Troubleshooting

Tubing Connections

Special attention must be paid to all tubing connections. Common mistakes in routine practice are:

- Nut and ferrule not connected tightly and leak
- Pilot distance incorrect
- Tubes not cut square
- Wrong type of ferrule or nut used
- Air bubbles trapped

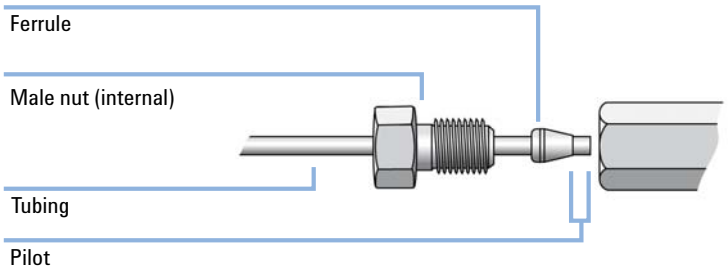


Figure 68 Definition of Terms for Tubing Connections

Disregarding these basic rules described below could lead to:

- Dead volume
- Peak deformation or peak splitting
- Carry-over effects
- Decreased resolution of the tubing between column and detector

Nut and Ferrule Tightening

Stepwise Tightening

CAUTION

Overtightening

Overtightening even once will damage the seat and the next connection can only be sealed by force. If the seat is damaged, there is a considerable chance that the ferrule will stick and cannot be removed without force or special tools (such as a drill).

If different materials are used, such as polymer tubing and nut together with a stainless steel ferrule, then over-tightening is always a danger. The stainless steel ferrule will bite into the polymer tube and block the flow.

➔ Do not over-tighten the nut/ferrule. If a connection proves to be leaky, use another quarter turn to tighten. Step-by-step tightening is the correct approach.

The first important rule when tightening a nut and ferrule is to proceed stepwise:

1. Tighten the nut by hand as much as possible.
2. Continue to tighten using a wrench until resistance is felt. Add one or two quarter turns to reach the sealing point.
3. Open the connection and remove the assembly to inspect the pilot distance, see Figure 68 on page 153.
4. Install the assembly again and tighten the nut manually at first. Use the wrench to reach the same sealing or resistance point as in the step before and add one extra quarter turn to obtain the final seal.
5. Check the seal when liquid is pumped through the system. In case a leak is observed, tighten once more by another quarter turn. The rule is to always go from leaking to properly sealed, and never try to release an over-tightened seal.

NOTE

When installing a tube with a narrow bend, typically a loop, it is advisable to fix one connection first without inserting the second nut into the female counterpart. After tightening, open the connection. Start preparing the second connection without inserting the first nut. Open the second connection again and check the pilot distance. The last step is to insert the complete tubing with the two connections and make a final seal as described above.

This stepwise installation of a bended tube is mandatory for PEEKsil tubing (fused silica tubing sheathed with PEEK polymer).

Re-use of Installed Nuts/Ferrules

NOTE Do not re-use an installed nut/ferrule for any other connection. Any tube with an installed nut/ferrule used for one purpose should not be installed for any other application.

1 Cut a portion of the tube end until straight tubing is reached and remove the ferrule.

NOTE This rule also applies to loops. Never re-use a loop with another valve or a valve after exchanging the stator.

NOTE If a finger-tight nut has been installed, replace it as well.

If a stainless steel nut has been used, ensure that the correct type is used, see "Wrong Nuts or Ferrules used" on page 159.

NOTE Newly installed finger-tight ferrules can hold a backpressure of up to 200 bar.

This remark is valid for the well known 'finger tight nuts' which have been used for a long time. Recent developments try to overcome this deficiency. Please observe the latest developments on the market to find a suitable solution.

Pilot Distance Incorrect

NOTE When making a new tube connection it is critical to follow these guidelines.

NOTE Failure to follow this procedure will lead to an incorrect pilot distance, which will result in actual dead volume (not delay volume).

1. Slip the nut and ferrule over the tube. The end of the tube should stick out approximately 50 mm (2 inch).
2. Press the tube end firmly into its female counterpart for the connection.
3. Move the nut and ferrule together into the female counterpart for connection. At this stage is it important to maintain pressure on the tube to avoid the tube slipping backwards, out of position.
4. Tighten the nut as described above.

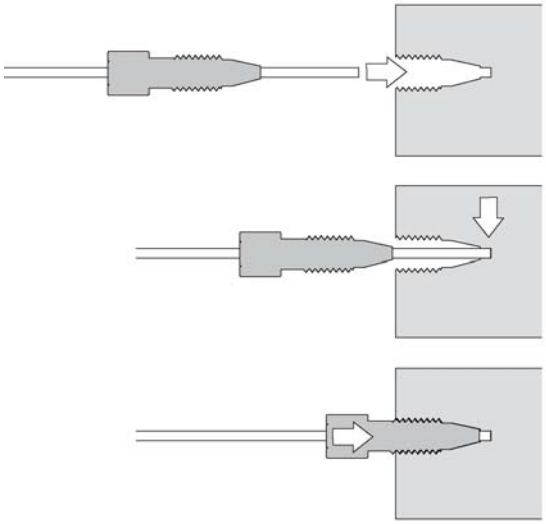


Figure 69 Pressing the Tube into Place

Tube Not Cut Square

All tubes must be cut absolutely square. Any deviation from square causes a dead volume which yields carry-over and other chromatographic effects. This applies to stainless steel, PEEK or Polymer tubing. It is important to use the correct tool for each material when cutting a tube.

- Stainless steel tubes are often cut using pliers. Often, an egg-shaped profile will result, which will no longer seal and will cause dead volume. Dedicated pliers for HPLC tubing are available; nevertheless, it is recommended to use precut tubing which is cut smooth, clean and passivated.
- Polymer tubes, such as PEEK, PTFE, PFA, etc., are soft and entice the user to cut them with any handy tool. Commercially available tubing cutters from many manufacturers are available. If the blade does not provide a clean, right-angle cut, use a different technique or tool.

The best and most commonly-used method for cutting tubing of any material is to use a cutter with an adjustable blade.

1 Carry out the initial turns.

2 Readjust the blade a very little.

3 Turn once more.

4 Adjust again until approximately half of the tubing wall is cut.

5 Hold the tube on both sides of the cut with flat-nose pliers.

6 Twist the tube until it breaks.

NOTE For polymer tubing, the same can be done by replacing the flat-nose pliers with tweezers with a flat tip.

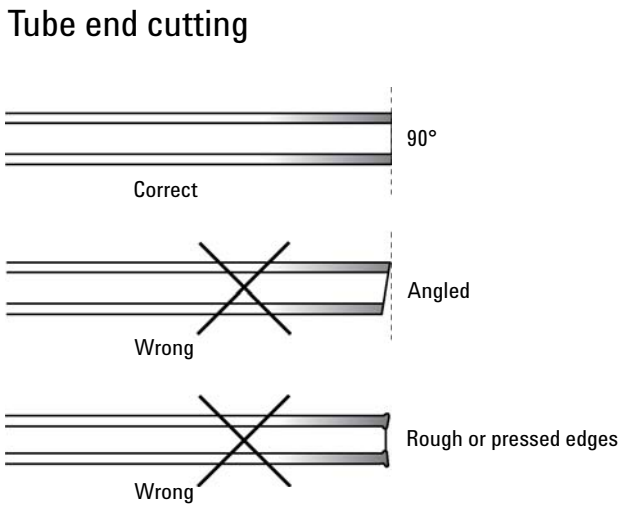


Figure 70 Illustration of various Tubing Cuts

Wrong Nuts or Ferrules used

Do not use a nut or ferrule from a vendor other than specified for the product. Figure 71 on page 159 illustrates the resulting dead volume that occurs when an incorrect type is used for connecting.

Figure 72 on page 159 shows the various forms of the ferrules from different manufacturers. Be aware that some of them are very similar in shape but not similar enough to interchange without risk.

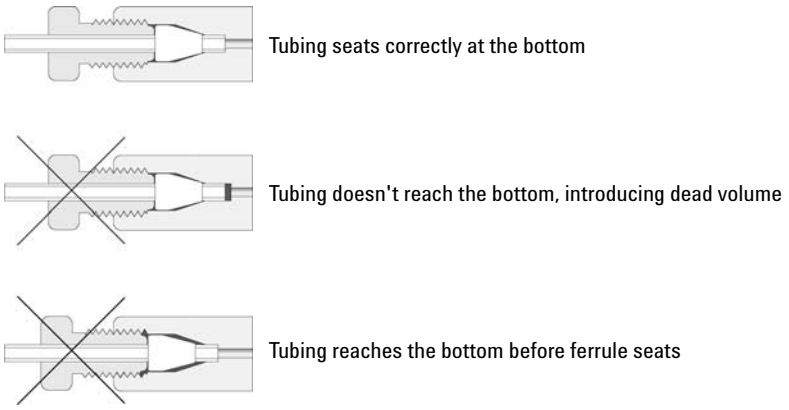


Figure 71 Dead Volume created due to wrong Ferrule Type

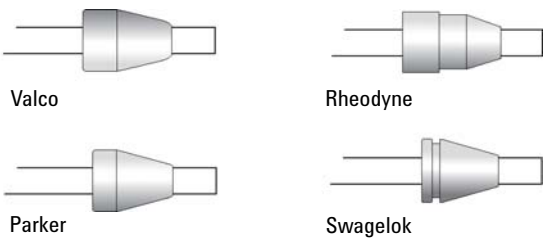


Figure 72 Ferrule Types from various Vendors

Avoiding Trapped Air Bubbles

It is common practice to make a connection only if the ports are wetted. The liquid helps to prevent trapped air bubbles which are often very difficult to remove or work out of a system.

Solvent Delivery Pumps are not priming

It has been observed that the solvent delivery pumps of the 'Pump Module' can fail to prime at initial operation.

The pump has to be wetted at the solvent inlet port, or preferably prime the tubing from the Solvent reservoir to the pump and make the connection.

Dry running the pump for a short time will not harm it.

Solvent Delivery Pump is not functioning

The pump flow rate depends greatly on the viscosity of the selected wash solvent and the given backpressure of the entire system. The valve bore size must be considered, as well as the tubing and injection loop internal diameter.

If in doubt about the delivered volume of a pump, a simple test can provide the answer.

Execute the first test, only if the result is negative is it necessary to check the flow from the pump directly.

Test 1: Pump flow delivered throughout the entire system

1 Prepare a graduated cylinder with a volume of 10 or 20 mL, Test solvent: Water.

2 Select the following path from the local Terminal: Menu/Utilities/Wash Station/select the desired Wash Station

The delivery pump and the DLW Actuator are activated, the delivered flow can be collected at the DLW syringe needle.

Expected result: A volume of approximately 8 mL should collect within 30 s (Water, DLW syringe needle Gauge 22).

Test 2: Pump flow delivered at pump outlet

1 Disconnect the outlet tube from the pump module.

2 Connect PEEK tubing with the following dimensions to the outlet of the pump module. Length 85 mm, ID 0.25 mm (0.010 inch).

3 The wash solvent in the reservoir bottle should be water; if necessary, replace the solvent.

4 Activate the pump by selecting following path:
Menu/F3-Setup/Objects/Events/Pwr-Out1 or Pwr-Out2.

5 Collect the pumped water in a graduated cylinder.

Within 30 s the pump should deliver approximately 10 mL.

This short test will give an indication of whether the system is performing as expected. If none or very little flow is observed, perform a systematic check using a process of elimination. Consult the table in "Troubleshooting" on page 146 for more details.

Verify the Functionality of the DLW Actuator/Solenoid

The DLW Actuator is electrically connected to the circuit of the heated syringe. Increasing the heated syringe temperature activates the DLW Actuator. The green LED is the indication that current is applied but please note that this is not proof that the DLW Actuator opens the seat to allow a solvent flow. To check the functionality, the following two tests are described:

Test 1: Electrical activation of the DLW Actuator

1 Select the DLW syringe using following path Menu | Utilities | Syringe | Standby Temp | and select the standby temperature 65 °C.

Expected result: The green LED on the Syringe Holder assembly is turned ON

In case this result is not observed, verify the electrical connection from the DLW Actuator to the PCB.

NOTE Do not reset the temperature if the second test is activated as well.

Test 2: Verify the seal tightness of the DLW Actuator

1 Close the DLW Actuator using the following path (from local Terminal): Menu | Utilities | Syringe | Standby Temp | and select standby temperature 35 °C.

Expected result: The green LED on the Syringe Holder assembly is turned OFF.

2 Place a beaker or other adequate vessel under the DLW Syringe Needle.

3 Select the Power-Out signal and activate both DLW Pumps. Path: Menu | 'F3-Setup' | Objects | Events | PwrOut1 for Pump1 , or 'PwrOut2' for Pump2

4 Activate both pumps by changing the status to the 'Active State' (Active State OFF = Pump is activated, start. Active State ON = Pump is stopped).

Expected result: The DLW Actuator has been closed (Step 1). Activating the DLW Pumps delivers solvent up to the point of the DLW Actuator. No solvent should pass the DLW Actuator which verifies the tightness of the seal.

NOTE In case a solvent flow is observed during the test, remove the DLW Actuator and check if particles are breaking the seal. If nothing special is observed, replace the device. The PNo. is listed in the Appendix.

Standard Chromatographic Tests

In this section standard chromatographic tests for 'carry-over' and 'repeatability' are described. These tests should provide basic information for support technicians and users.

The tests described are examples only and should be adjusted accordingly if the configuration of the HPLC system changes.

The test is designed for standard valves with a bore size of 0.25 mm, which allows a flow rate range of 10 to 500 µL/min.

For both the carry-over and repeatability tests, conditions will be described for UV detection.

Details are listed below. Note that the given levels to reach for carry-over and repeatability do not reflect the specification values. The tests described here should give the operator a confirmation whether the system is working as expected. These tests are comparable to an OQ (Operational Qualification) Test.

The test procedure is written for standard equipment, such as a standard syringe of 100 µL and a loop size of 20 µL. The procedure does allow configuring the system with different equipment, but the user has to bear in mind certain limitations. For example, if a 100 µL syringe is installed alongside a loop with a volume < 10 µL, the reliability of a partial loop injection is questionable. The relative standard deviation will be above the expected limit of 1.20 %. Example: 2 µL loop, 1 µL partial loop filling yields a relative standard deviation of 2 – 4 %. Under such conditions, a full loop injection is the only valid test.

Carry-Over Test with UV-Detection

- Test Sample: Chlorhexidine, 0.6 mg/mL dissolved in Water : Acetonitrile = 90 : 10 + 0.1 % TFA
- Blank Solution: Water : Acetonitrile + 0.1 %TFA
- HPLC System:
  - Binary Solvent Delivery Pump
  - UV-Detector, Wavelength: 257 nm
  - HPLC Column: Zorbax SB-C18, 2.1 x 50 mm; spherical 1.8 µm (or equivalent)
  - Column Temperature: Ambient
  - Mobile Phase: A: Water + 0.1 % TFA; B: Acetonitrile + 0.1 % TFA
  - Gradient:
    - 10 % A hold for 0.5 min
    - to 90 % B in 7.5 min
    - hold for 1 min
- Wash Solvents:
  - Wash1: Water + 0.1 % TFA
  - Wash2: Acetonitrile + 0.1 % TFA
- Loop Size:
  - 2 µL; Loop ID 0.13 mm
- Injection Valve: Agilent 1200 bar injection valve 5067-4123
- Cycle: DLW Standard
- Method Parameter Settings

Table 33 Carry-Over Test with UV-Detection

Parameter	Value	Parameter	Value
Syringe	100 µL DLW	Pullup Del	500 ms
Sample Volume	7 µL	Inject to	Specified Inj. Valve
Airgap Volume	3 µL	Inject Speed	5 µL/s
Front Volume	5 µL	Pre Inj Del	500 ms
Rear Volume	5 µL	Pst Inj Del	500 ms
Needle Gap Valve Clean	3 mm	Valve Clean Time Solvent 2	2 s
Fill Speed	5 µL/s	Post Clean Time Solvent 2	2 s
Fill Strokes	0	Valve Clean Time Solvent 1	2 s
Stator Wash	0	Post Clean Time solvent 1	2 s

Repeatability Test with UV-Detection

- Test Sample
  - Loop Overfill Caffeine, 520 mg/L
  - Partial Loop Caffeine, 52 mg/L dissolved in Water : Acetonitrile = 90 :10 + 0.1 % TFA
- HPLC System
  - Binary Solvent Delivery Pump
  - UV-Detector, Wavelength: 273 nm
  - HPLC Column: Zorbax SB-C18, 2.1 x 50 mm; spherical 1.8 µm (or equivalent)
  - Column Temperature: Ambient
  - Mobile Phase: Water : Acetonitrile = 90 :10 + 0.1 % TFA
  - Gradient: isocratic for 2 min
- Wash Solvents:
  - Wash1: Water + 0.1 % TFA
  - Wash2: Acetonitrile + 0.1 % TFA
- Loop Size: 2 µL (Loop overfill, 7 µL); Loop ID: 0.13 mm; 20 µL (partial Loop, 10 µL); Loop ID 0.25 mm
- Injection Valve: Agilent 1200 bar injection valve 5067-4123
- Method Parameter Settings

Table 34 Repeatability Test with UV-Detection

Parameter	Value	Parameter	Value
Syringe	100 µL DLW	Pullup Del	500 ms
Sample Volume	7 µL/10 µL	Inject to	Specified Inj. Valve
Airgap Volume	3 µL	Inject Speed	5 µL/s
Front Volume	5 µL	Pre Inj Del	500 ms
Rear Volume	5 µL	Pst Inj Del	500 ms
Needle Gap Valve Clean	3 mm	Valve Clean Time Solvent 2	2 s
Fill Speed	5 µL/s	Post Clean Time Solvent 2	2 s
Fill Strokes	0	Valve Clean Time Solvent 1	2 s
Stator Wash	0	Post Clean Time solvent 1	2 s

Carry-Over and Repeatability Tests with UV-Detection

- Test Sample:
  - Benzophenone 50 µg/mL dissolved in Water : Methanol = 50 : 50
- Blank Solution:
  - Water : Methanol = 20 : 80
- HPLC System:
  - Binary or isocratic Solvent Delivery Pump
  - UV-Detector, Wavelength: 258 nm (10 mm path length)
  - HPLC Column: C18 , 2.1 x50 – 100 mm spherical 2.7 – 5.0 µm (or equivalent)
  - Column Temperature: Ambient to 30 °C
  - Mobile Phase: A: 20 % Water; B: 80 % Methanol
  - Isocratic: 0.5 – 1.0 mL/min
- Wash Solvents:
  - Wash1: Water : Methanol = 50 : 50
  - Wash2: Water : Methanol = 50 : 50
- Loop Size:
  - 20 µL; Loop ID 0.25 mm
- Injection Valve:
  - Agilent 1200 bar injection valve 5067-4123
- Cycle:
  - DLW Standard
- Method Parameter Settings

Table 35 Test with Benzophenone and UV-detection

Parameter	Value	Parameter	Value
Syringe	100 µLDLW	Pullup Del	5000 ms
Sample Volume	60 µL	Inject to	Specified Inj. Valve
Airgap Volume	3 µL	Inject Speed	5 µL/s
Front Volume	5 µL	Pre Inj Del	500 ms
Rear Volume	5 µL	Pst Inj Del	500 ms
Needle Gap Valve Clean	3 mm	Valve Clean Time Solvent 2	6 s
Fill Speed	2 µL/s	Post Clean Time Solvent 2	6 s
Fill Strokes	0	Valve Clean Time Solvent 1	6 s
Stator Wash	0	Post Clean Time solvent 1	6 s

Replacing Parts

Warnings and Cautions

WARNING

High voltage

Capacitors inside the instrument may still remain charged even if the instrument is turned off.

- ➔ Always disconnect the power cord(s) from the power supply or from the various power supplies if optional devices are installed before attempting any type of maintenance.
- ➔ To avoid damaging electrical parts, do not disconnect an electrical assembly while power is applied to the PAL system. Once the power is turned Off, wait approximately 30 seconds before you disconnect an assembly.

Replacing Control Board

Parts required	#	p/n	Description
	1	G6500-64220	PCB, Main for G427xA, G6501B, G6509B, AGI2

NOTE The Control board combines the MOTIO and the CPU boards in a single control board. This board is required for the HTS System and operates only at Firmware level 4.0.X.

1 Follow the sequence shown in Figure 73 on page 172 to detach and release the Control board from its position inside the X-axis.

   a As shown, remove the dummy cover first.

2 Carefully pull the two ribbon cable connectors upwards to free them from the board.

3 Install the replacement board in the reverse order.

NOTE After reconnecting the two ribbon cables to the board, ensure that the cables are folded flat and can slide inside the X-axis without damage.

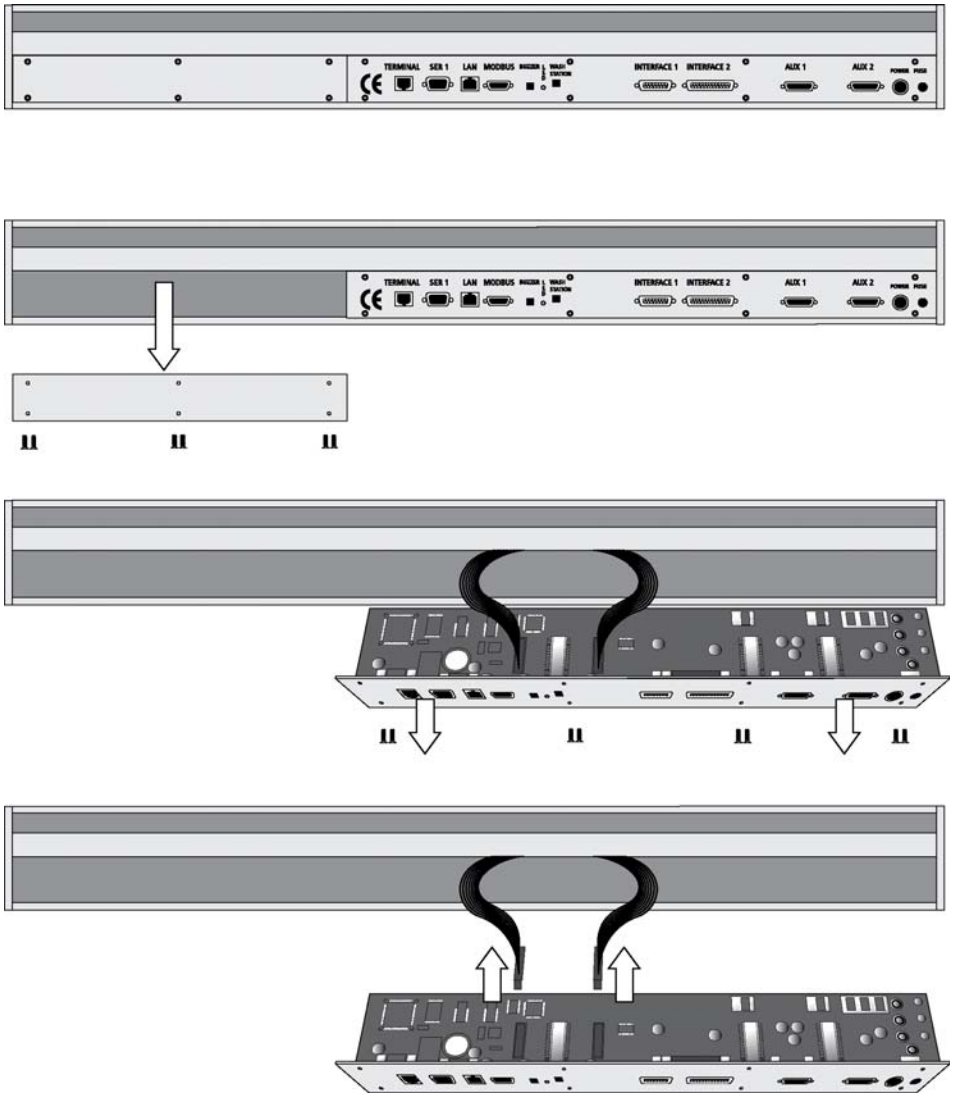


Figure 73 Replacing Control Board (HTS)

Injection Valve / Valve Rotor

1 After the mechanical connection of the PAL Valve Drive to the X-axis and electrical connection to AUX1 at the board, turn on the power of the PAL System.

   The Firmware Object for the Valve type **PAL VlvDrv-5067-4123_1** is preloaded, the Valve Drive will go into the Standby position.

2 Insert the valve body into the shaft until the pin engages in the shaft.

3 Center the valve body by turning the valve body into position until the vertical port is aligned.

4 Insert the Valve Body completely into the shaft until the pin on top of the valve engages in the threaded Valve Drive Bracket. Tighten the Valve Body with the polymer connecting nut.

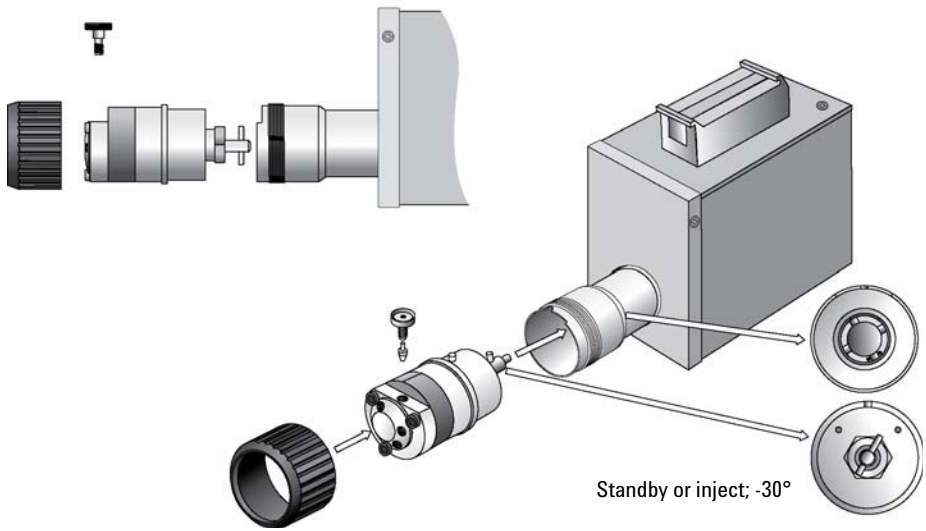


Figure 74 Valve Installation

Injection Unit

Follow the sequence of steps shown in "Assembling the LC Injector HTC/HTS" on page 55.

DLW Pump Module

The following parts (keywords) from the DLW Pump Module can be exchanged individually:

p/n	Description
G4277-60510	DLW Pump Module (re-order for one solvent channel)
G6500-88035	Solvent Bottle Transfer Line including Glass Filter Solvent Inlet, 40 µm pore size
G4277-60513	Wash Station cable to System
G4277-60511	Pump control PCB
G4277-60512	DLW Tubing Kit

DLW Wash Station

The following parts (keywords) from the DLW Wash Station Module can be exchanged individually:

p/n	Description
G4277-60514	DLW Wash Station incl. Waste Tube
G4277-60515	Needle Guide Length Tool
G4270-67205	Waste Tube
G4277-60517	Waste Tube (Valve to Wash station)

DLW Syringe

The following parts (keywords) from the DLW Syringe assembly can be exchanged individually:

p/n	Description
G4277-80120	DLW Syringe 100 µL
G4277-80122	DLW Syringe Plunger (for syringe 100 µL)
G4277-60509	DLW Plunger Holder

DLW Syringe Holder Assembly

The following parts (keywords) from the DLW Syringe Holder assembly can be exchanged individually:

p/n	Description
G4277-60601	DLW Syringe Holder Assembly (complete assembly)
G4277-60604	DLW Needle Holder Assembly
G4277-60602	DLW Holding Loop (installed in the DLW Syringe Assembly)
G4277-60503	DLW Manifold (Entire unit assembled, consisting of DLW Solvent Selector, DLW Syringe Adapter with Perfluor O-ring installed.)
G4277-60505	DLW Actuator/Solenoid
G4277-60603	DLW Actuator Control PCB (incl. cable)
G4277-60507	DLW Syringe Holder Fixation Screw

Replacing the DLW-2 Holding Loop

The DLW-2 Holding Loop is a combined part with injection needle and holding loop as one piece. The holding loop is made out of stainless steel and the inside is passivated with acid.

After a certain period of use or if mechanical damage occurs (for example bent needle) the holding loop may need to be replaced.

Acid passivation is often used in HPLC technique to remove substances which have accumulated over time. Typically a 20 % aqueous solution of nitric acid or phosphoric acid is used.

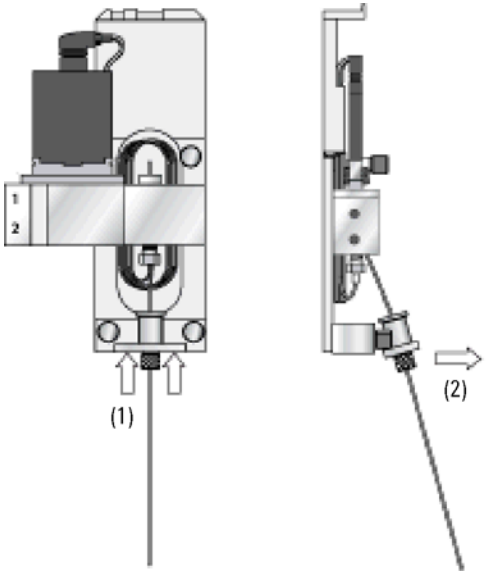
It is highly recommended to periodically (for example once a year or more often if the application requires it) clean critical parts which are always in contact with the sample solution, such as the holding or the injection loop.

Please note that tubing, nuts, ferrules or any other part in the HPLC system flow path made out of PEEK or another polymer may have resistance problems against concentrated mineral acids.

1 Remove the PAL DLW-2 Syringe Holder Assembly from the Z-axis.

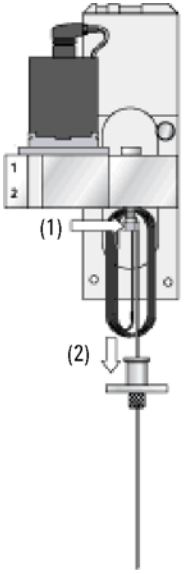
2 Remove the DLW Syringe and check at same time the condition of the glass barrel and plunger; check tightness of the plunger.

3 Push up the DLW Needle Holder Assembly (1) and click it out of the DLW Needle Adapter Block (2).

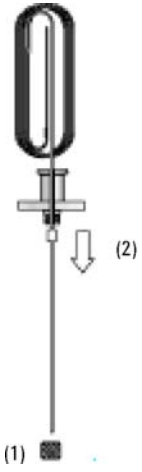


4 Unscrew the Needle Holder Block.

5 Unscrew the retaining nut which tightens the holding loop to the DLW Manifold (1). Pull out the DLW Holding Loop (2).



6 Pull down the DLW-2 Holding Loop and remove it from the DLW Syringe Holder. Release the retaining nut from needle (1). Move the holding loop down to be able to disengage the lower part of the holding loop (Needle) from DLW Needle Holder Assembly (2).



7 Installing a new DLW-2 Holding Loop: Install a new loop in reverse order, from Step 7 to Step 1.

DLW Option Spare Parts

Spare Parts Order Information for DLW-2 Option

NOTE

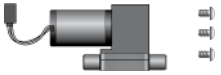
This section of Spare Parts is intended for the DLW-2 Option only.

For Spare Parts for the previous version, DLW Option, see "Spare Part Order Information for DLW Option" on page 183

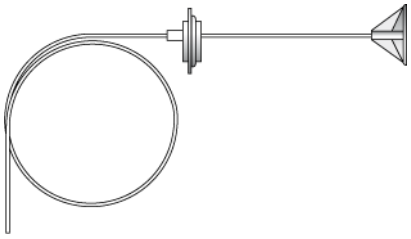
For upgrade path from 'DLW' to 'DLW-2', see "Upgrade Path to DLW-2 Option" on page 185.

DLW Pump Module

p/n	Description
G4277-60510	DLW Pump Kit (single module); incl. electrical connections; 24 V



G6500-88035	Aspiration Tube Kit, consisting of: 1 pc. Tube PFA 1 pc. Glass Filer, 40 µm pore size 1 pc. Plug with feed through hole



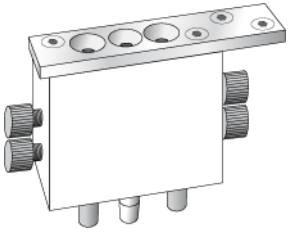
G4277-60513	Cable for -xt Wash Station round connector, length 130 cm

p/n	Description
G4277-60511	DLW Pump Control Module PCB Kit

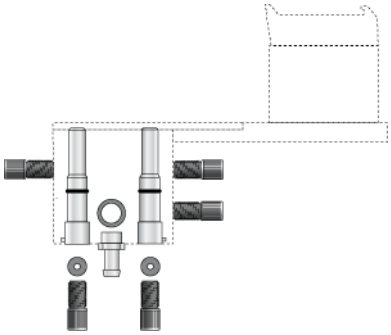
G4277-60512	DLW Wash Station Tubing Kit, Consisting of: Tubing set, wire supports and connections

DLW Wash Station

p/n	Description
G4277-60514	DLW Wash Station Block, complete assembly (incl. centering plate)



p/n	Description
G4277-50515	DLW Wash Station Insert Kit, consisting of: 2 pcs Inserts 1 pc. Waste Connector 5 pcs. Dummy Plugs



G4270-67205	Tube Waste for Wash Station, length 2 m

DLW Syringe

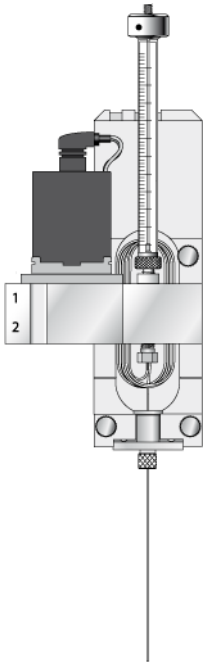
p/n	Description
G4277-80120	Syringe for DLW Option, 100 µL gastight; Ø7.7 mm; Scale Length 60 mm; Thread ¼ - 28 UNF. Removable needle not included.

G4277-80122	Replacement Plunger for Syringe SYRC DLW-R.; pkg of 10.

G4277-60509	DLW Plunger Holder

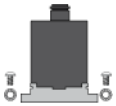
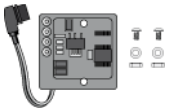
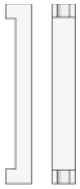
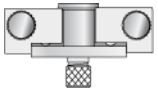
DLW Syringe Holder Assembly

p/n	Description
G4277-60601	DLW-2 Syringe Holder Assembly; complete Assembly with Holding Loop and 100 µL Syringe installed



G4277-60602	Kit DLW-2 Holding Loop with Needle, Adapter mounted, including Retaining Nut

G4277-60503	DLW Manifold Kit, complete, assembled

p/n	Description
G4277-60505	PAL DLW Actuator/Solenoid Kit; incl. screws, without cable 
G4277-60603	DLW-2 Actuator Control PCB Kit; incl. cable and screw set 
G4277-60507	Syringe Holder Fixation Screw, pkg of 1 
G4277-60516	DLW Needle Length Guide Tool, pkg of 1 
G4277-60604	DLW-2 Needle Holder 

Spare Part Order Information for DLW Option

NOTE

This section of Spare Parts is specific to the DLW Option.

The listing below shows only the spare parts which are specific to the 'DLW Option'. The other parts are either identical to the 'DLW-2 Option Spare Parts' or are backwards compatible.

The upgrade path from 'DLW' to 'DLW-2' is described under "Upgrade Path to DLW-2 Option" on page 185.

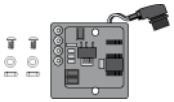
DLW Syringe

p/n	Description
G4277-80125	Needle Kit for DLW Option, Gauge 22 PST 3, length 51 mm (3 pcs. per pack); incl. Needle Retaining Nut.

DLW Syringe Holder Assembly

p/n	Description
G4277-60502	Kit DLW Holding Loop with Needle, Adapter mounted

G4277-60504	DLW Flow Diverter Pkg of 1

p/n	Description
G4277-60506	DLW Actuator Control PCB Kit; incl. cable and screw set. 'DLW' version not available as single spare part anymore. The 'DLW-2' version is not backwards compatible. Select 'Upgrade Holder', PNo.: DLW-2UpgrdKit. See "Upgrade Path to DLW-2 Option" on page 185. 
G4277-60508	DLW Needle Holder Kit 'DLW' version not available as single spare part anymore. The 'DLW-2' version is not backwards compatible. Select 'Upgrade Holder', PNo.: DLW-2UpgrdKit. See "Upgrade Path to DLW-2 Option" on page 185. 

Upgrade Path for DLW Option to DLW-2 Option

General Considerations

As described in "Development of the PAL DLW Option" on page 64, the DLW technique has undergone several development steps. The current version, DLW-2 Option, contains several improvements:

- Holding Loop

  The DLW-2 Holding Loop is one single piece from the needle to the loop. This eliminates several tube connections, the replacement needle and the Flow diverter.

  The loop is made of high quality stainless steel and the inner surface is passivated with acid.

- DLW Syringe Holder Back-plate

  The DLW-2 Syringe Holder back-plate has changed in size and form to allow a smoother replacement of the holding loop. The Actuator is supported from the back side for greater mechanical stability.

- DLW Pump Assembly

  The DLW-2 Pump assembly is newly equipped with a housing to protect the electrical parts from solvent splashes.

Upgrade Path to DLW-2 Option

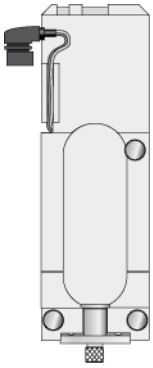
The following parts are not backwards compatible with the older DLW Option:

p/n	Description
G4277-60603	DLW Actuator Control PCB (incl. cable)
G4277-60604	DLW Needle Holder Assembly

If one of these parts has to be replaced, is an upgrade kit available.

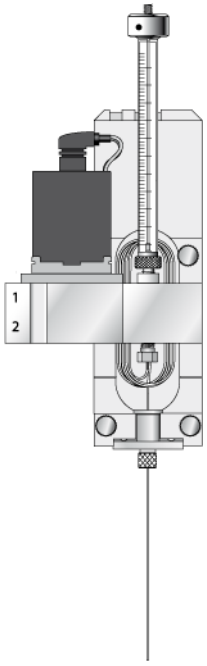
This kit is based on the DLW-2 version of syringe holder plate with installed Actuator PCB and Needle Holder.

p/n	Description
G4277-60605	PAL DLW-2 Upgrade Kit Contains: DLW-2 Syringe Holder Plate, PAL DLW Actuator Control PCB, PAL DLW Needle Holder



If one wishes to upgrade the entire DLW Holder to take advantage of the mechanical changes and the stainless steel holding loop, is recommended to use following order information:

p/n	Description
G4277-60601	DLW-2 Syringe Holder Assembly; complete Assembly with Holding Loop and 100 µL Syringe installed.



Comparison DLW and DLW-2 Option

The following table compares the critical parts or parameters of the 'DLW' and the 'DLW-2' Options. The specifications for the parts of the DLW-2 Option are listed in "Specifications" on page 48.

Table 36 Comparison Parts and Parameters of the 'DLW' and 'DLW-2' Options

Part or Parameter	DLW Option	DLW-2 Option
Holding Loop		
Material	FEP Tubing	Stainless Steel, passivated
OD	1/16" (1.58 mm)	0.72 mm (Gauge 22)
ID	0.50 mm	0.41 mm
Length	550 mm	855 mm
Volume	108 µL	118 µL
Syringe Needle	51 mm	Not applicable, integrated in
Length	22	DLW-2 Holding Loop
Gauge	Stainless steel	
Material		
DLW Flow Diverter	20.5 mm	Not applicable, integrated in
Length	0.72 mm (Gauge 22)	DLW-2 Holding Loop
OD	0.41 mm	
ID	Stainless Steel	
Material		
DLW Internal Volume	205 µL + Injection Loop volume (204.7)	208 µL + Injection Loop volume
Total Delay Volume	Manifold: 90 µL	Manifold: 90 µL
	Holding Loop : 108 µL	Holding Loop: 118 µL
	Syringe Needle: 6.7 µL	Installed Injection Loop
	Installed Injection Loop	

Maintaining the LC Injector HTC/HTS System

Regularly performing maintenance helps ensure accuracy and precision of the autosampler System. Suggested intervals for maintenance procedures are given below to ensure smooth, uninterrupted operation. If you use the autosampler system extensively (for example, throughout nights and weekends), or if you use corrosive solvents, you may need to perform the maintenance procedure more frequently.

Table 37 Maintenance Checklist

Maintenance Step	Interval
Clean the outside of the instrument. Use only a soft lint-free cloth dampened with mild soap and water.	Weekly or as needed
Clean Instrument, syringe and surfaces	Weekly or as needed.
Replace the syringe plunger	The Syringe Plunger for a gas tight syringe (polymer tip) has to be replaced on a regular basis. The interval is highly dependent on the application, throughput, quality of sample solution (particles, etc.) and washing solvent. It is advisable to check the tightness of the plunger on a regular basis to gain experience with the application.
Replace the Valve Needle Seal	Check the tightness of the needle in the needle seal. See "Valve Needle Guide" on page 112.
Replace the Valve Rotor	Annually or more often, depending on the throughput and quality of sample solution and mobile phase.
Wash and Waste Solvent reservoirs. Check flow restriction of frits. Check quality of solvents, presence of biological growth.	Weekly or as needed. Replace the Septum as well to avoid carry-over.
Prime the entire DLW system by using the '**Utilities**' functions '**Wash Stations**' or '**Syringe**'.	Daily or as needed. Required before routine running.

Table 37 Maintenance Checklist

Maintenance Step	Interval
Replace the DLW Syringe plunger.[1]	The Syringe Plunger for a gas tight syringe (polymer tip) has to be replaced on a regular basis. The interval is highly dependent on the application, throughput, quality of sample solution (particles, etc.) and washing solvent. **NOTE** It is advisable to check the tightness of the plunger on a regular basis to gain experience with the application.
Replace the DLW Syringe Needle (in one piece with DLW-2 Holding Loop).	Only as needed (e.g. if bent).
Check all connections for tightness.[1]	Weekly or as needed.
Replace the DLW Holding Loop.[1]	Recommended to be changed if an excessive carry-over effect is observed and cleaning of the Holding Loop does not improve the situation.
Check the spring loaded Needle Holder Assembly for functionality. Pay special attention to the spring compression. [1]	Weekly or as needed.
Check the functionality of the DLW Actuator/Solenoid.[1]	Weekly or as needed. Recommended to be changed once a year as a preventative maintenance step. See "Verify the Functionality of the DLW Actuator/Solenoid" on page 162

[1] Dedicated to the DLW Option

NOTE There are no operator-serviceable or replaceable parts inside the power supply(ies) or the autosampler System. In case of any failure, contact a representative of Agilent Technologies.

4 Appendices

This chapter provides addition information.

Definition of Terms

Job Queue

A Job Queue is a list of sample processing Jobs. Jobs are executed in the order displayed on the JOB QUEUE menu screens. New Jobs may be added to the queue while samples are being processed.

Job

A Job contains the information needed by the LC Injector HTC/HTS to process multiple samples by the same processing steps. The elements of a Job are a Method and a Tray that define the location of the samples to be processed. For identification, Jobs are automatically numbered from 01 to 99 and then restarting with 01 when they are added to the Job Queue.

Cycle

A Cycle consists of the specific operations necessary to process one sample. The Cycle operations are repeated for each sample within a Job. Cycles are designed for specific applications.

Method

A Method defines how the samples are processed. The elements of a Method are a Cycle, a Syringe and a Parameter List. Methods have names with up to eight characters and can be edited, copied, and deleted.

Method Parameters

Method Parameters are associated with the Cycle operations. User-assigned Parameter values define how a processing operation is performed. A zero Parameter value will disable a Cycle operation. Cycle Parameters are application-specific.

Tray Holder

A Tray Holder can hold one or more trays. Each Tray Holder has a reference position (X-, Y-, Z-coordinates) that defines its location.

Tray

A Tray holds multiple samples. Trays are defined by designating the Tray Type (see below) and the Tray Holder. Tray names are used to identify the sample source within a Job.

Tray Type

A Tray Type defines the pattern and sampling sequence of sample locations within a Tray.

Stack

A Stack is a particular type of Tray Holder that is designed to hold micro-plates. A six-drawer Stack holds 12 standard microplates, two in each drawer.

A three-drawer Stack holds six deep-well micro-plates, two in each drawer.

Object Manager

Software to load an Object List to an instrument if a Module (hardware module) has been added to the System. In a special mode Object Manager can also be used to create and maintain Object Lists.

Object List

If a Module (hardware) is added to an instrument, several Objects have to be loaded into the firmware. These Objects are collected in an Object List and stored in a file with the extension "*.pol".

Object Lists are delivered together with Object Manager Software and are grouped into folders for the different kind of Modules (e.g. Syringes, Tray Holders, Valve Drives). The name of an Object List starts with the Module part number with variants added (e.g. first or second Stack). The name of the root folder includes the revision which is dependent on the firmware version (e.g. "**Object Lists Rev. K**" for firmware 2.X and 3.X).

Object Class

Each Object belongs to an Object Class (e.g. Syringes, Trays, Injectors).

The Object Class defines the Items of an Object.

Object Item

An Object contains several Items which can be numerical values with a physical unit (e.g. X-, Y-, Z-Position, Penetration, Syringe Scale Length, Syringe Volume) or references to other objects.

NOTE The term “Parameter” is reserved for “ATOM Parameter” (Firmware commands to be used for a Cycle).

Objects

Objects are data structures describing the properties of physical modules. Certain modules (e.g. a Stack) require several objects.

Module

Hardware module, either part of a standard system configuration (e.g. Agilent LC Injectors HTC/HTS, Agilent GC Sampler 80/120) or an optional addition (e.g. SPME Upgrade for Agilent GC Injector, MT/DW Tray, Stack, and Cooler Upgrades for Agilent LC Injectors HTC/HTS). The term “Module” is intentionally used to differentiate from “Object”, which is reserved for the Firmware Object.

Conventions of Naming

This section recommends standard naming convention for LC Injector HTC/HTS Trays, Tray Types, and Tray holders. Following these conventions will allow LC Injector HTC/HTS to be pre-configured for certain applications, will simplify software backups and application development, and will improve technical support and training.

Table 38 Naming Convention

Tray Type	Tray Description
VT200	Vial Tray, 200 positions (10 x 20) For 7 mm micro-vials, 1 ml
VT98	Vial Trays, 98 positions (7 x 14) For 12 mm vials, 2 ml
VT78	Vial Tray, 78 positions (6 x 13) For 7 mm micro-vials, 1 ml (opposite side of 98 positions Tray)
VT54	Vial Tray, 54 positions (6 x 9) For 12 mm vials, 2 ml
VT21	Vial Tray, 21 Positions (7 x 14) For 12 mm vials, 2 ml (opposite side of 32 positions Tray)
VT32-10	Vial Tray, 32 positions (4 x 8) For 23 mm headspace vials, 10 ml
VT32-20	Vial Tray, 32 positions (4 x 8) For 23 mm headspace vials, 20 ml
MT96	Standard 96-position shallow microplate
DW96	Deep well 96-position microplate
MT384	High density 384-position shallow microplate

Injection Cycles

"LC-Inj" Cycle

No.	Processing Step	Method Parameter	Object	(Class)	Used Object Value
1	Reference X, Y, Z axes and syringe plunger			(System)	Start Ref = ON
2	Wait for start signal		**Start**	(Sync Signals)	
3	Read Barcode if enabled		**TRAY**	(Tray Type)	Barcode ? None
4	Syringe wash strokes with solvent from Wash1	**Pre Cln Slv1**	**SYRINGE** **Wash1**	(Syringes) (Wash Stations)	Fill Speed Eject Speed Needle Penetr Clean Volume Max Fill Speed Max Eject Speed
5	Syringe wash strokes with solvent from Wash2	**Pre Cln Slv2**	**SYRINGE** **Wash2**	(Syringes) (Wash Stations)	Fill Speed Eject Speed Needle Penetr Clean Volume Max Fill Speed Max Eject Speed
6	Rinse syringe with sample selected number of times	**Pre Cln Spl** **Sample Volume** **Fill Speed** **Pullup Delay**	**SYRINGE** **TRAY** **Waste**	(Syringes) (Tray Type) (Injectors)	Eject Speed Fill Volume Needle Penetr Needle Penetr

No.	Processing Step	Method Parameter	Object	(Class)	Used Object Value
7	Fill syringe with sample using several filling strokes; aspirate air outside of vial if Air Volume > 0	**Sample Volume** **Air Volume** **Fill Speed** **Fill Strokes** **Pullup Del**	**SYRINGE** **TRAY**	(Syringes) (Tray Type)	Eject Speed Overfill Fill Volume Needle Penetr
8	Move to injection valve	**Inject to**			
9	Wait for Inject signal		**Inject**	(Sync Signals)	
10	Drive needle to penetration depth	**Inject to**		(Injectors)	Needle Penetr
11	Delay	**Pre Inj Del**			
12	Switch valve to Active position				
13	Activate Injected signal		**Injected**	(Out Signals)	Pulse Time
14	Activate Injectd+ Signal		**Injectd+**	(Out Signals)	Pulse Time
15	Dispense syringe contents into sample loop	**Inject Speed**			
16	Delay	**Pst Inj Del**			
17	Switch valve to Standby position				
18	Retract needle from injector				
19	Clean syringe and needle with solvent from Wash1	**Pst Cln Slv1**	**SYRINGE** **Wash1**	(Syringes) (Wash Stations)	Fill Speed Eject Speed Needle Penetr Clean Volume Max Fill Speed Max Eject Speed

No.	Processing Step	Method Parameter	Object	(Class)	Used Object Value
20	Clean syringe and needle with solvent from Wash2	**Pst Cln Slv2**	**SYRINGE** **Wash2**	(Syringes) (Wash Stations)	Fill Speed Eject Speed Needle Penetr Clean Volume Max Fill Speed Max Eject Speed
21	Rinse valve inlet with solvent from Wash1	**Vlv Cln Slv1**	**SYRINGE** **Wash1**	(Syringes) (Wash Stations)	Fill Speed Eject Speed Needle Penetr Clean Volume Max Fill Speed Max Eject Speed
22	Rinse valve inlet with solvent from Wash2		**SYRINGE** **Wash1**	(Syringes) (Wash Stations)	Fill Speed Eject Speed Needle Penetr Clean Volume Max Fill Speed Max Eject Speed
23	Move to Home position		**Home**	(Positions)	

"LC-Inj" Cycle Parameter Description

Parameter	Description
CYCLE	LC-Inj Cycle
SYRINGE	Selected syringe
Sample Volume	Selected sample volume
Air Volume	Volume aspirated after the syringe needle is moved out of the sample liquid
Pre Cln Slv1	Pre injection syringe wash strokes with solvent from Wash1
Pre Cln Slv2	Pre injection syringe wash strokes with solvent from Wash2
Pre Cln Spl	Pre injection syringe rinse cycles with sample
Fill Speed	Speed of plunger movement used to aspirate sample
Fill Strokes	Number of filling strokes to aspirate sample
Pullup Del	Delay time between sample pull-up and ejection
Inject to	Name of Injector used for injection
Inject Speed	Speed of plunger movement used during sample injection
Pre Inj Del	Delay time prior sample injection
Pst Inj Del	Delay time after sample injection
Pst Cln Slv1	Post injection syringe wash strokes with solvent from Wash1
Pst Cln Slv2	Post injection syringe wash strokes with solvent from Wash2
Vlv Cln Slv1	Valve rinse cycles with solvent from Wash1
Vlv Cln Slv2	Valve rinse cycles with solvent from Wash2

DLW Principles and Operating Details

Priming the Solvent Lines

The priming of the DLW Option is conveniently done directly from the local terminal. There are several tasks which can be executed from the '**Utilities**' section. It is recommended to use the function of the Wash station first, followed by '**Clean Syringe**'.

Follow this description step-by-step to prime the solvent lines and the entire DLW system. Solvent degassing and filtering is recommended. Important is to observe the sequence of steps to ensure that Solvent 1 will be the last solvent in the DLW System. Good practice is to have the wash solvent with lower chromatographic strength (elution power) defined as '**Wash Solvent 1**' and '**Wash Solvent 2**' consistently as the solvent with a stronger elution power.

NOTE For trouble-free DLW operation, make sure the two solvent lines are free of air bubbles at all times. If the solvent lines are being connected for the first time or during a solvent change, it is necessary to prime the solvent lines properly until air bubbles are no longer visible. Solvent degassing and filtering is recommended.

Priming with Utilities Function 'Wash Station'

Prime the PAL DLW System with the Utilities function 'Wash Station'

1 Select **Menu > Utilities > Wash Station**.

2 Select **Wash 2 > Function Key 'F3' > 'Move to Wash' > Function Key 'F2' > 'Activate Valve'**

3 Observe the green LED as an indicator that the DLW Actuator is activated. Prime with Solvent 2 until no further air bubbles are observed.

4 Repeat the steps to prime with Solvent 1, by activating Wash 1.

Priming with Utilities Function 'Syringe'

As second priming step for the PAL DLW System, the Utilities function '**Syringe**' is recommended. This function allows control of the plunger movement.

NOTE It is important that the priming through '**Wash Station**' is completed and that the solvent lines are completely free of air bubbles.

1 Select **Menu > Utilities > Syringe > Function Key 'F2' 'Cln Syr'**.

2 Select **Wash 2**

   a Select teh desired '**Clean Count**' (recommended a minimum of 3 counts)

3 Observe the green LED as an indicator that the DLW Actuator is activated and ensure that the plunger moves smoothly. Prime with Solvent 2 until no further air bubbles are observed.

4 Repeat the steps to prime with Solvent 1, by activating Wash 1.

Other Priming functions or Troubleshooting

Another priming function is available in the section **Utilities > Injector**.

1 Select '**Clean Injector**'

2 Follow the dialog with similar logic as described under "Priming the Solvent Lines" on page 200

In case any problems are observed, follow the instructions given in "Troubleshooting" on page 146. Dedicated tests to verify the DLW functionality are described in this section.

Location of Solvent and Waste Bottles

The DLW Option contains self-priming membrane pumps. The solvent bottles can be placed either in the Fast Wash Station Holder or on the lab bench.

The Waste bottle must be placed >30 cm below the injection valve. Make sure that the waste liquid can flow into the waste bottle without restriction. The waste tubing must be placed above the level of the liquid. Ideally, the tube should be fixed at the neck of the waste bottle.

NOTE

Use good lab practice to avoid contaminating wash solvents and bottles. Avoid the growth of biological contaminants in pure water by either replacing it regularly or adding a small amount of organic solvent, such as methanol or acetonitrile. Certain buffer solutions can decompose at room temperature when exposed to light. Filtering the wash solvents before filling the bottles, especially if salt buffers are used, is mandatory to avoid clogging of the solvent paths.

Functionality of the DLW Option

DLW Pumps

From the control point of view, the DLW pumps respond in the same manner as the Fast Wash Station. The pumps are activated by '**Power-out Signals**'. The electric current setting for the DLW is different; this means that the corresponding Firmware Objects have to be loaded for each DLW wash station type. See "Loading the LC Injector HTC/HTS DLW Object" on page 98 for further details.

The wetted parts in the pump are made of the following materials:

- Membrane: Kalrez (FFPM)
- Body, Valves: Ryton PPS

The pumps are self priming with a suction lift of up to 3 m water column.

DLW Actuator/Solenoid

The '**DLW Actuator/Solenoid**' separates and completely shuts off the lines in the direction of the syringe (sample loading) and the wash solvent.

After opening the DLW Actuator/Solenoid for the wash solvent lines, the desired wash solvent can be pumped into the system by activating the corresponding DLW pump.

The schematic in Figure 75 on page 203 illustrates this function.

The wetted parts in the DLW Actuator Solenoid are made of the following materials:

- Solenoid body: PEEK
- Seal material: FFKM (Simriz)

NOTE PEEK exhibits excellent chemical resistance to most commonly used chemicals. However, the following solvents are not recommended for use with PEEK, DMSO, THF, methylene chloride (dichloromethane), nitric acid, sulfuric acid. For more details see the compatibility tables provided by the manufacturer of PEEK materials or components.

NOTE In cases of defects, such as a defective or clogged Actuator/Solenoid, the LED would light up but this does not mean that the solvent path is opened.

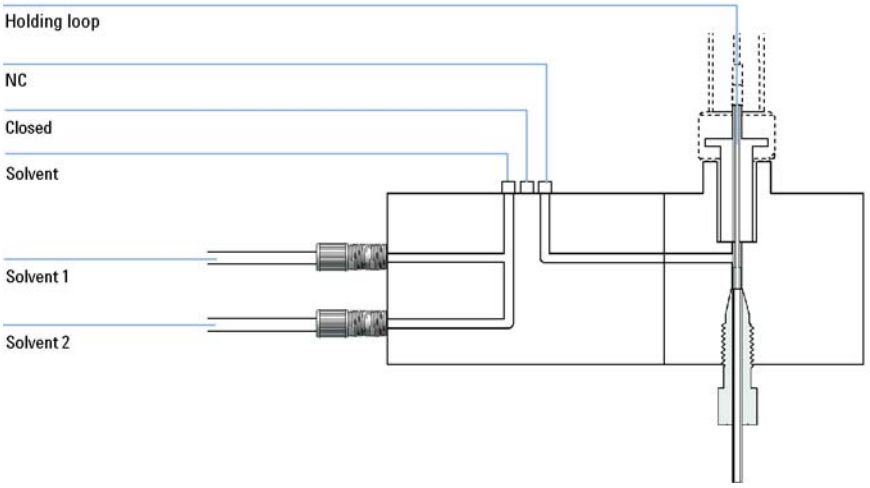


Figure 75 DLW Manifold and Actuator/Solenoid

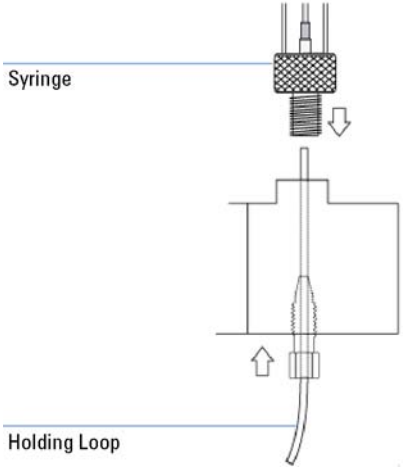


Figure 76 Schematic of DLW Manifold with Holding Loop

ICC Cycles used for the DLW Option

The DLW Option can only be operated in combination with Agilent ChemStation, Masshunter and EZChrom data systems.

For software control two cycles are provided. The first cycle is used for **Standard** speed with optimized washing possibilities, and the second for a **Fast** cycle with optimized throughput and less focus on carry-over (see Table 39 on page 204).

The two cycles are explained in detail, see Table 40 on page 205 and Table 41 on page 208.

Table 39 DLW Cycle Definitions

Cycle Name	Cycle Description
LC-Inj DLW Standard	Standard injection cycle using all possibilities of the DLW Option. The injection valve inlet port and the needle are washed with both wash solvents (inside and out). An extra '**Stator Wash**' can be added for intensive washing of the injection valve (valve toggle).
LC-Inj Fast	The cycle is tuned for speed and high-throughput applications. The difference with regard to the Standard DLW cycle is that some steps are left out to shorten the cycle time. Details are explained below.

General Considerations

The duration of the wash steps has to be established for each configuration and application. The viscosity and surface tension of the individual wash solvents composition and the backpressure of the system are factors to consider.

Be aware that a higher backpressure builds up if the valve bore size or the installed loop internal diameter are lower (standard valve bore 0.25 mm). The standard loop internal diameter (ID) for loops provided with a volume of 5 , 10 and 20 µl is 0.25 mm. The loop with 2 µl content volume has an ID of 0.125 mm.

Keep the tubing internal diameters of the tubing in line with the valve dimensions, loop ID and flow rate.

Standard DLW Injection Cycle

Cycle Name: LC-Inj DLW Standard Rev03

Table 40 LC-Inj DLW Standard

Cycle Description	Cycle Variable
The System waits for the Sync Signal **Ready** before the injection cycle is started.	**NOTE** Sync Signal setting **Start** Figure 77 on page 212
The Injection Valve is brought to a defined position: **Standby**.	Inject to Standby
The **Rear** air segment is pulled into the Holding Loop.	Airgap Volume Filling Speed Figure 78 on page 213
The combined volumes of **Rear-**, **Sample List-**, and **Front-Volume** are aspirated into the Holding Loop. The **Front** air segment is aspirated.	Front Volume Figure 79 on page 214 Rear Volume (SL.volume) Airgap Volume Filling Speed Pullup Delay Figure 80 on page 215
The Injection Unit moves to the DLW Wash station, position **Wash1**. The needle is inserted (dipped) for 1 second to wash the outer needle surface. No plunger movement occurs at this step.	Figure 81 on page 216
The Injection Unit moves to the specified Injection Valve. The **Front-** and **Airgap-Volume** are ejected.	Inject to Front Volume Airgap Volume Injection Speed Figure 82 on page 217
The System waits for the signals from the data system	**Wait for DS**

Table 40 LC-Inj DLW Standard

Cycle Description	Cycle Variable
The Injection Valve is switched to position **Active**. The system pauses according to the delay time **Pre Inject Delay**. The loop is filled with the sample volume as specified in the sample List. The Injection Valve is switched to position **Standby**, the loop contents are injected. Timer 1 (**Delay Stator Wash** is started and a **Start** signal to the HPLC system is sent.	Inject to Pre Injection Delay Figure 83 on page 218 (SL.volume) Injection Speed Post Inject Delay Timer 1 Figure 84 on page 219
The plunger of the DLW Syringe is pushed down to dispense the **Rear Sample** and **Air Segment** to Waste. The Holding Loop is still filled with **Wash Solvent 1**.	(Syr. Eject Speed) Figure 85 on page 220
The DLW Actuator/Solenoid is activated to deliver **Wash Solvent 2** into the Holding Loop to clean the Injection Valve from Port 1 to Port **Waste**. For this step the needle tip is lifted, releasing the sealing pressure to enable rinsing around the tip sealing point. **NOTE** The Atom **Rinse Inj** is now available starting with Firmware 4.1.x. The DLW Actuator/Solenoid is activated, the Wash Solvent (pump), the **Needle Gap** and the **Rinse Time** are selectable.	Wash2 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 2 Needle Gap is a parameter from **Rinse Inj** Atom. the variable in this macro is: **Needle Gap Valve Clean**. The function of this parameter is to lift the needle in the injection port to allow rinsing around the needle tip. This pressure of the spring loaded balls in the DLW Syringe Holder Assembly is released by moving approximately 3 mmup (default). This leaves a gap between the needle tip and the valve bottom of approximately 1 mm, to enable a flush at this contact point. Figure 86 on page 221
The Injection Unit is moved to the DLW Wash Station, position **Wash2**. The needle is rinsed inside and out with **Wash Solvent 2**.	Wash2 (Syr.Eject Speed) Post Clean Time Solvent 2 Figure 87 on page 222
The Injection Unit is moved back to the Injection Valve. The Inlet Port and engraving to waste Port is flushed with **Wash Solvent 1** to prepare the valve for the next injection.	Wash1 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 1 Figure 88 on page 223

Table 40 LC-Inj DLW Standard

Cycle Description	Cycle Variable
The Injection Unit is moved back to the DLW Wash Station, position **Wash1** to flush the syringe needle inside and out with **Wash Solvent 1**. This is a preparation step for the next injection. Especially important for biofluid samples.	Wash1 Post Clean Time Solvent 1 Figure 89 on page 224
Cycle end for **LC-Inj DLW Standard** macro.	Figure 90 on page 225
An optional cleaning step is attached to the **DLW Standard** injection cycle: Stator Wash or Valve Toggle. A **Repeat-End** loop can be activated with the **Count**.	Stator Wash Stator Wash count: 1 Stator Wash count: 1 = Cleaning step active Stator Wash count: 0 = Cleaning step disabled
If **Stator Wash** is activated, the following steps will be executed.	
The Injection Unit is moved to the Injection Valve. From the last step above, the Holding Loop is filled with **Wash Solvent 1**. Timer 1 is awaited to switch the valve (Toggle) into **Active** position (fill loop).	Inject to Delay Stator Wash (Active) "Stator Wash: Steps 1 – 2" on page 229
The DLW Actuator/Solenoid is activated to deliver **Wash Solvent 2** to the Holding Loop and into the valve system. The first solvent flush arriving at the valve is **Wash Solvent 1** followed by **Wash Solvent 2**.	Inject to Wash2 Stator Wash Time Solvent 2 "Stator Wash: Step 3" on page 230
The Wash Solvent is changed to **Wash Solvent 1**.	Inject to "Stator Wash: Step 4" on page 231 Wash1 Stator Wash Time Solvent 1 "Stator Wash: Step 5" on page 232
The Injection Valve is switched back to the **Standby** position.	Inject to (Standby) "Stator Wash: Step 6" on page 233

Fast DLW Injection Cycle

The "**Fast**" injection cycle differs from the "**Standard**" cycle in three points:

- The needle is not dipped in the Wash station "**Wash1**" after sample pickup and before moving to the Injection Valve.
- The wash steps after injection are reduced to "**Valve Clean**" with Wash Solvent 1 and Wash Solvent 2. The DLW needle is flushed in the DLW Wash Station with "**Wash Solvent 1**" only.
- "**Stator Wash**" (Valve Toggle) is not available.

Cycle Name: LC-Inj Fast Rev03

Table 41 LC-Inj Fast Rev03

Cycle Description	Cycle Variable
The System waits first for the Sync Signal **Ready** before the injection cycle is started.	NOTE Sync Signal setting **Start** Figure 98 on page 234
The Injection Valve is brought to a defined position: **Standby**.	Inject to Standby
The **Rear** air segment is pulled into the Holding Loop.	Airgap Volume Filling Speed Figure 99 on page 235
The combined volumes of **Rear**-, **Sample List**-, and **Front-Volume** are aspirated into the Holding Loop. The Front air segment is aspirated.	Front Volume Figure 100 on page 236 Rear Volume (SL.volume) Airgap Volume Filling Speed Pullup Delay Figure 101 on page 237
The Injection Unit moves to the specified Injection Valve. The **Front**- and **Airgap-Volume** is ejected to **Waste**.	Inject to Front Volume Airgap Volume Injection Speed Figure 102 on page 238

Table 41 LC-Inj Fast Rev03

Cycle Description	Cycle Variable
The System waits for a signal from the data system	**Wait for DS**
The Injection Valve is switched to position **Active**. The system pauses according to the delay time **Pre Inject Delay**. The loop is filled with the sample volume as specified in the sample List. The Injection Valve is switched to position **Standby**, the loop contents are injected.	Inject to Pre Injection Delay (SL.volume) Figure 103 on page 239 Injection Speed Post Inject Delay Figure 104 on page 240
The plunger of the DLW Syringe is pushed down to dispense the **Rear Sample** and **Air Segment** to Waste. The Holding Loop is still filled with **Wash Solvent 1**,	Injection Speed Figure 105 on page 241
The DLW Actuator/Solenoid is activated to deliver **Wash Solvent 2** into the Holding Loop to clean the Injection Valve from Port 1 to Port 'Waste'. For this step the needle tip is lifted, releasing the sealing pressure to enable rinsing around the tip sealing point. **NOTE** The Atom '**Rinse Inj**' is new, available starting with FW 4.1.X. The DLW Actuator/Solenoid is activated, the Wash Solvent (pump), the '**Needle Gap**' and the '**Rinse Time**' are selectable.	Inject to Wash2 Needle Gap Valve Clean Valve Clean Time Solvent 2 Needle Gap is a parameter from '**Rinse Inj**' Atom. The variable in this macro '**Needle Gap Valve Clean**'. The function of this parameter is to lift the needle in the injection port to allow rinsing around the needle tip. The pressure of the spring-loaded balls in the DLW Syringe Holder Assembly is released by moving approx 3 mm up (default). This leaves a gap between the needle tip and the valve bottom of approx. 1 mm, to enable a flush at this contact point. **NOTE** The **Needle Gap Valve Clean** is explained in detail in the macro **DLW Priming**. Figure 106 on page 242

Table 41 LC-Inj Fast Rev03

Cycle Description	Cycle Variable
Wash Solvent 1 follows to prepare the valve for the next injection.	Wash1 Inject to Needle Gap Valve Clean Valve Clean Time Solvent 1 Figure 107 on page 243
The Injection Unit is moved back to the DLW Wash station, position **Wash1** to flush the syringe needle inside and out with **Wash Solvent 1**. This is a preparation step for the next injection. Especially important for biofluid samples.	Wash1 Post Clean Time Solvent 1 Figure 108 on page 244
Cycle end for **LC-Inj DLW Fast** macro.	Figure 109 on page 245

DLW Cycle Step-by-Step

NOTE The following illustrations display a valve configuration with a loop mounted between port 3 and port 6. For the Agilent 1200 bar injection valve the general principles for the DLW operation is the same, although the loop is mounted between port 2 and port 5.

Standard Cycle

Standard: Cycle Start

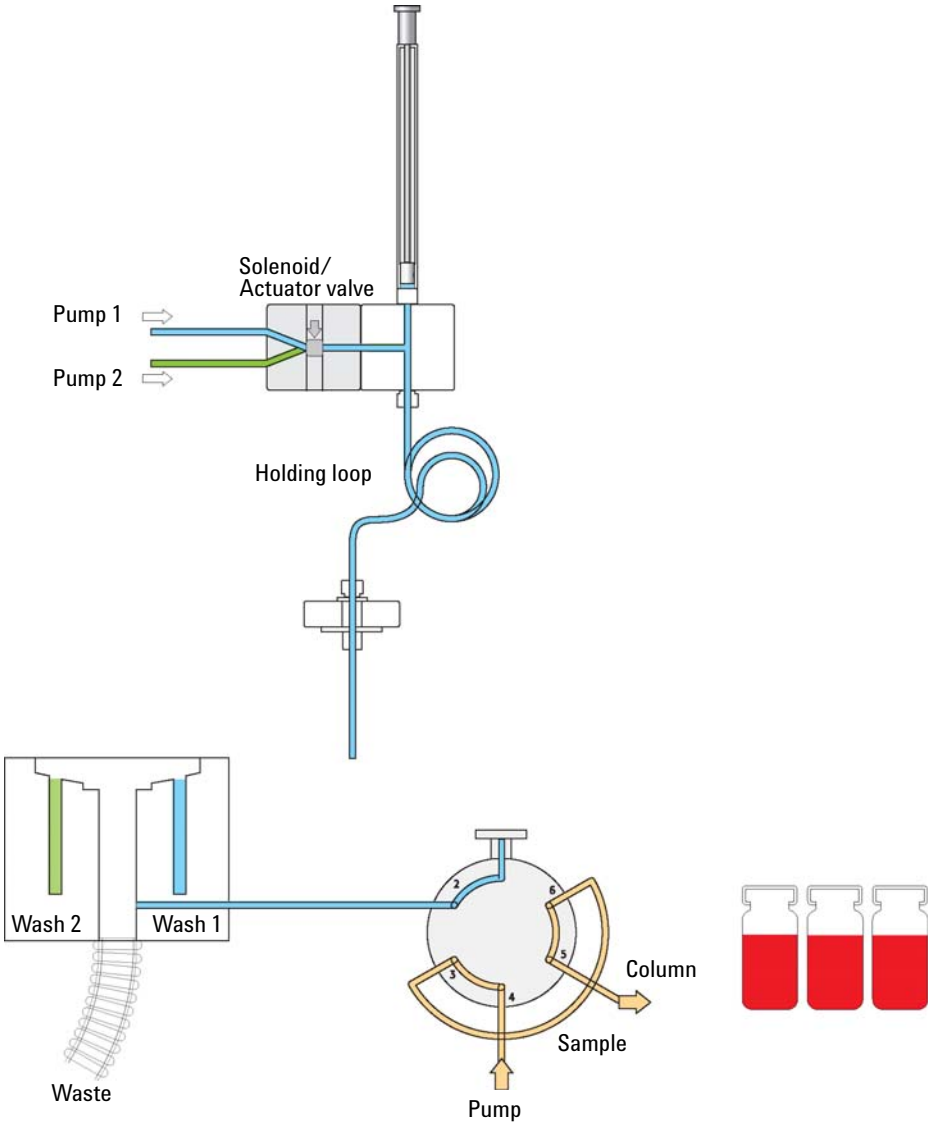


Figure 77 Standard Injection Cycle Start

Standard: Step 1

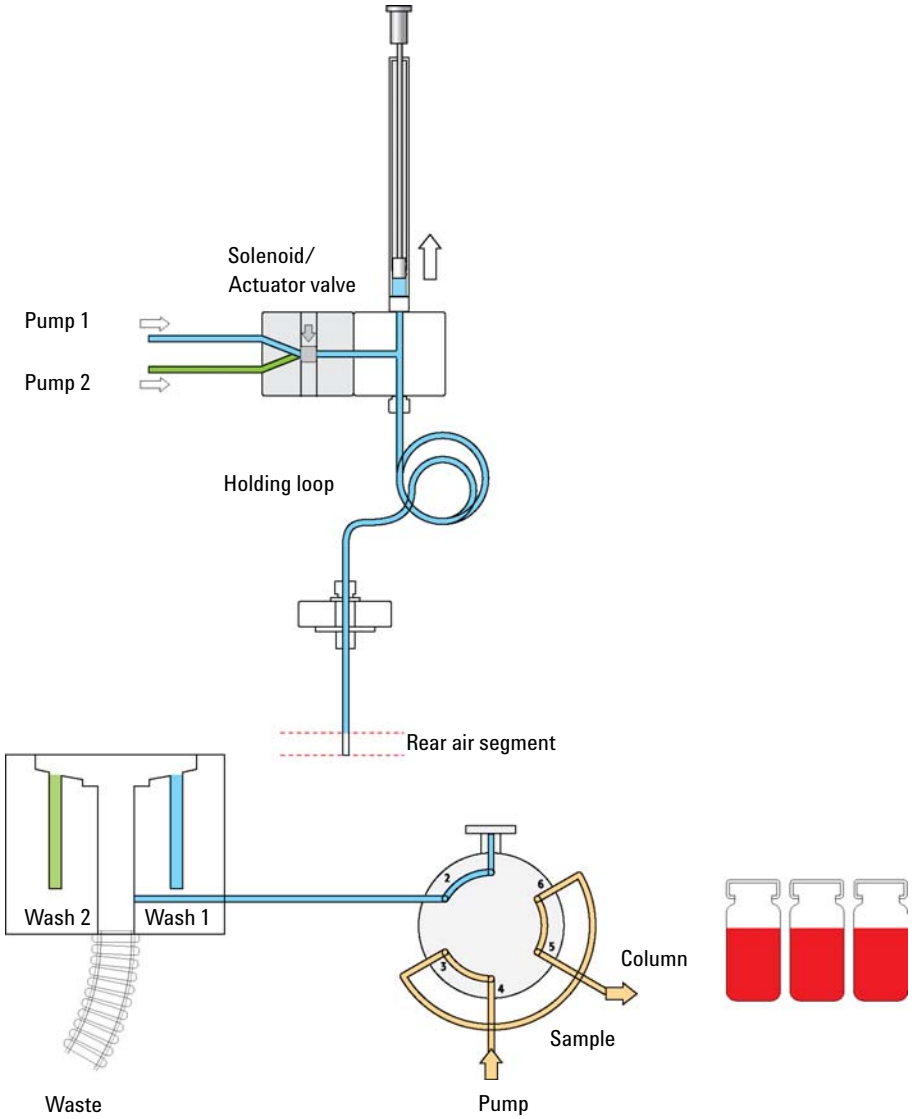


Figure 78 Standard Injection Cycle Step 1: Aspirate Rear Air Segment

Standard: Step 2

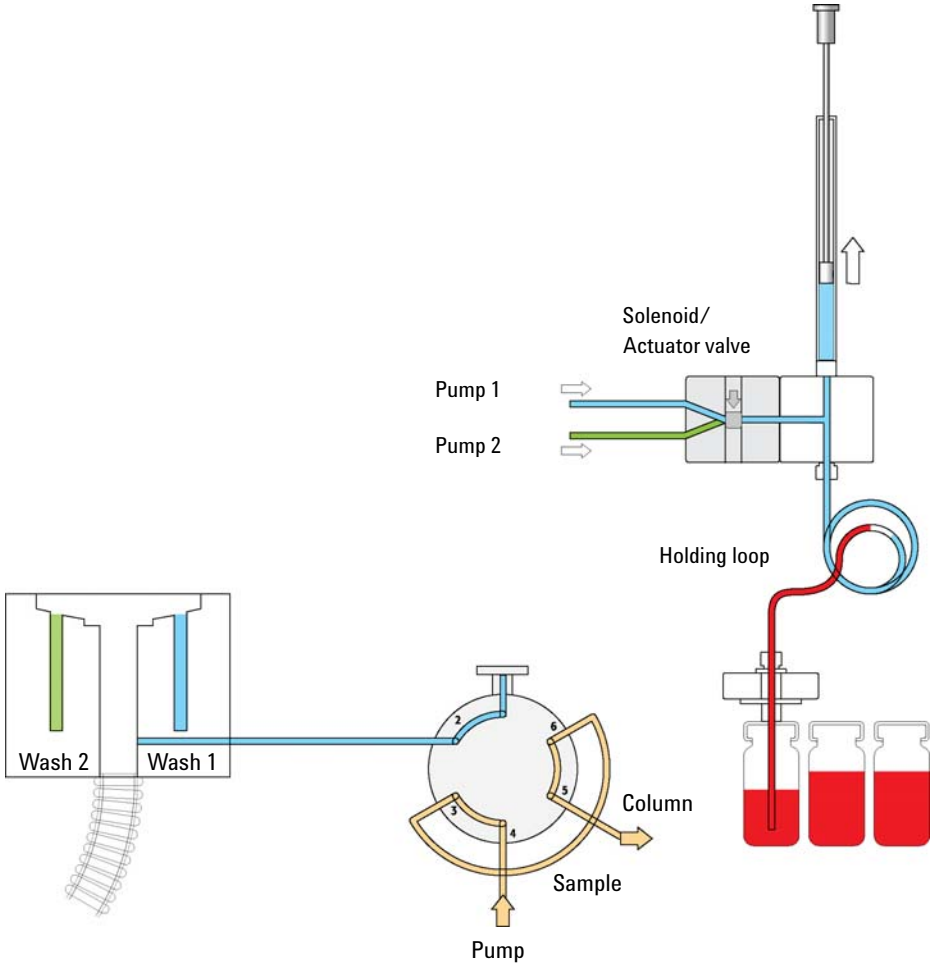


Figure 79 Standard Injection Cycle Step 2: Get Sample Aspirate Rear, Inject and Front Volume

Standard: Step 3 — 4

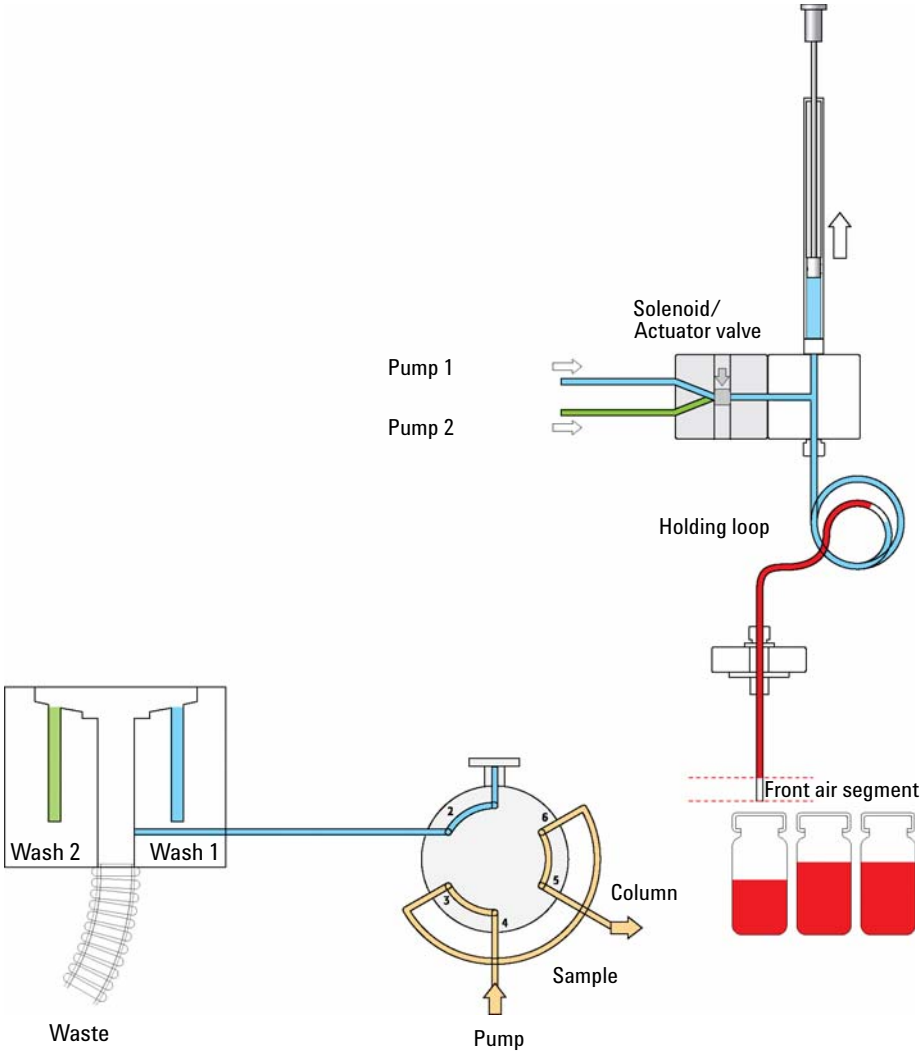


Figure 80 Standard Injection Cycle Step 3-4: Aspirate Front Air Segment

Standard: Step 5 — 6

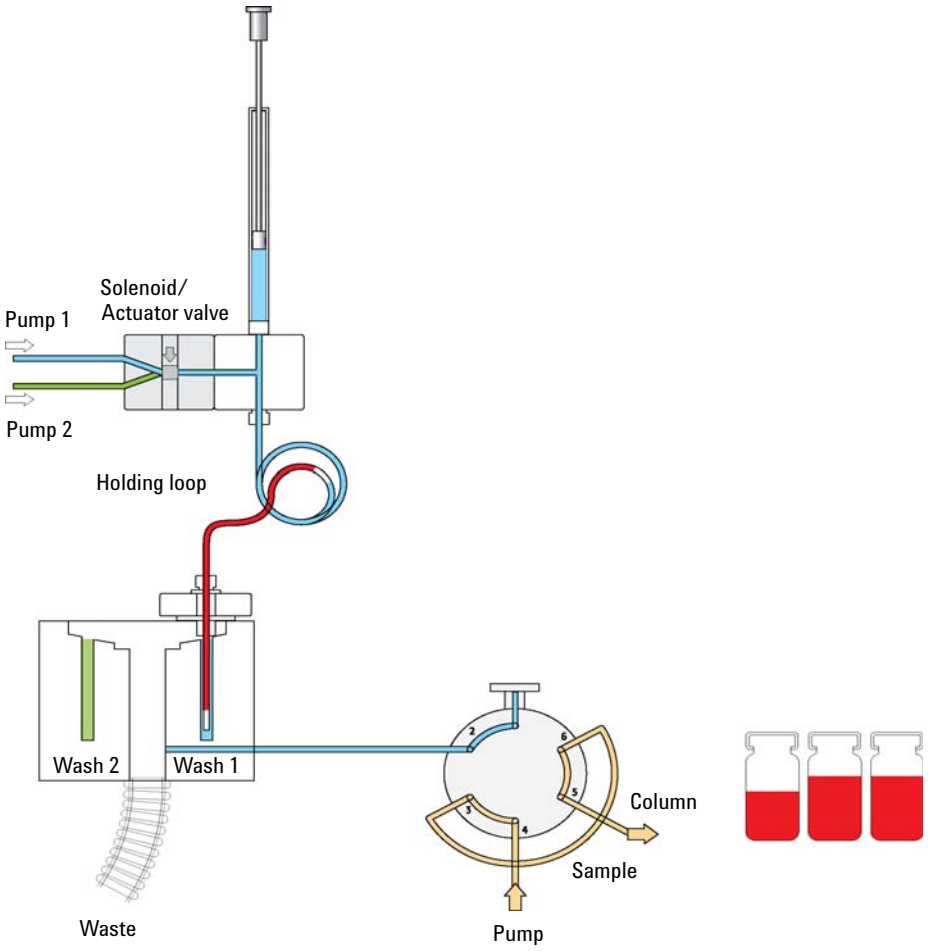


Figure 81 Standard Injection Cycle Step 5-6: Passive needle clean outside (dip) in wash position 1

Standard: Step 7 — 8

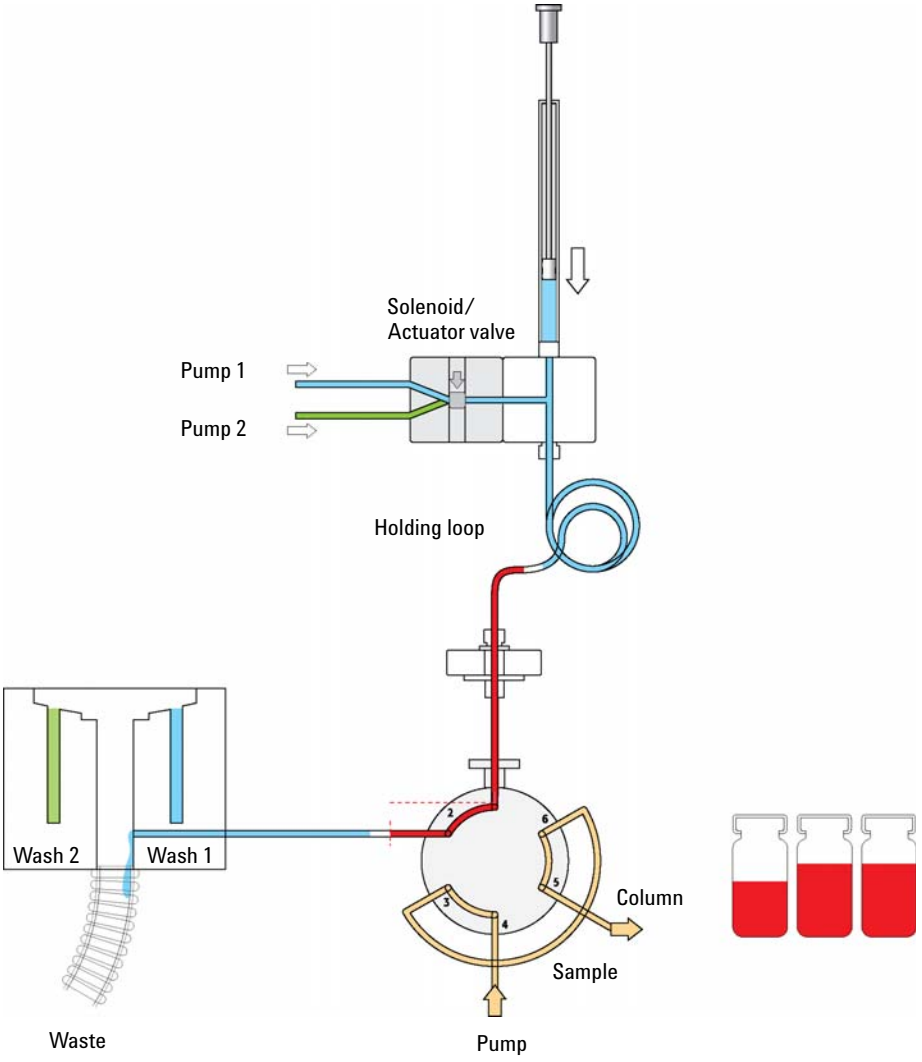


Figure 82 Standard Injection Cycle Step 7-8: Dispense Front Air Segment and Front Sample Volume to Waste

Standard: Step 9 — 10

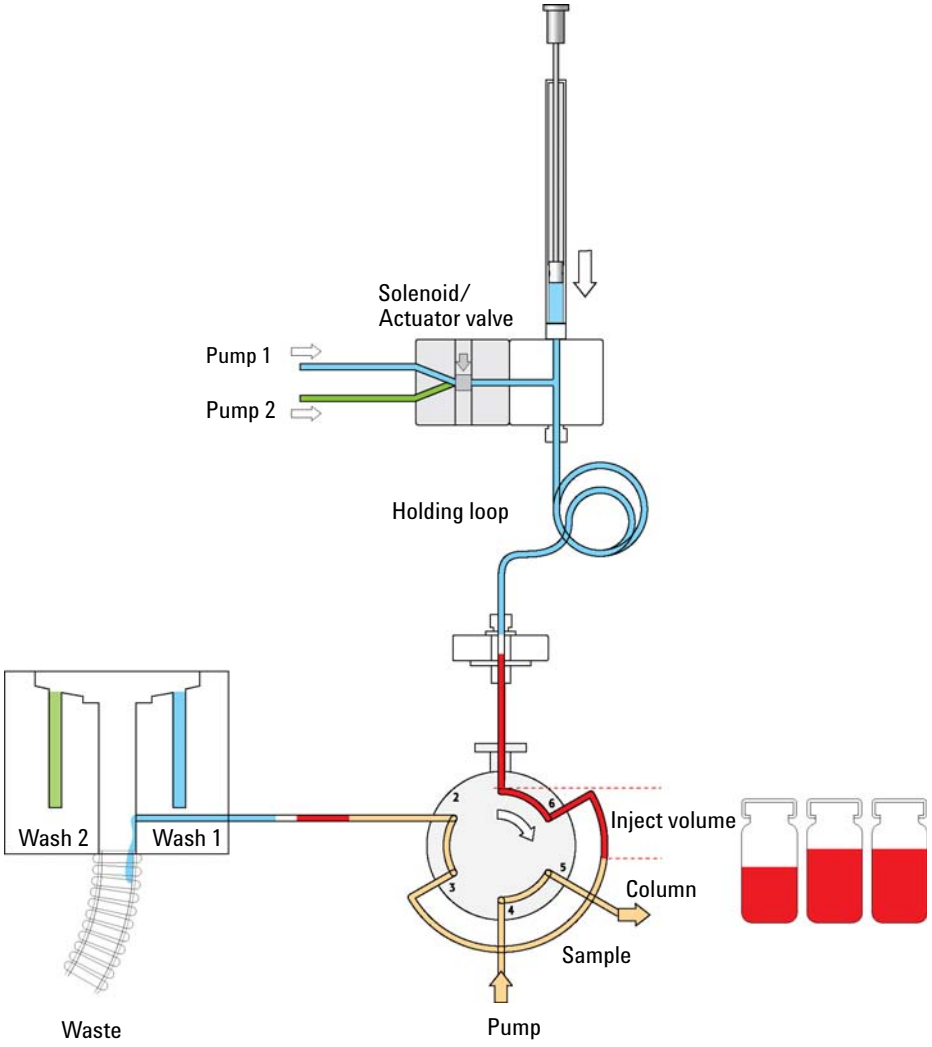


Figure 83 Standard Injection Cycle Step 9-10: Valve is switched to LOAD position, Loop is filled with 'Inject Volume'

Standard: Step 11

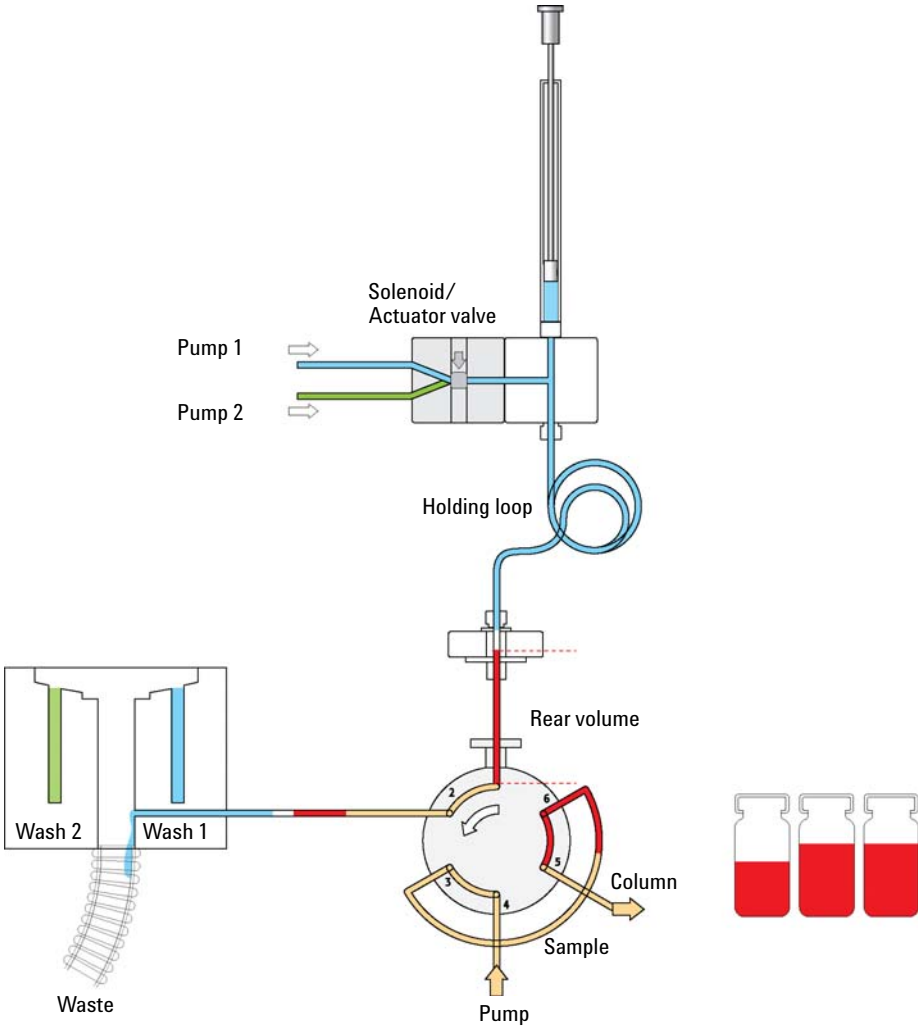


Figure 84 Standard Injection Cycle Step 11: Valve is switched to INJECT position, start chromatographic process

Standard: Step 12

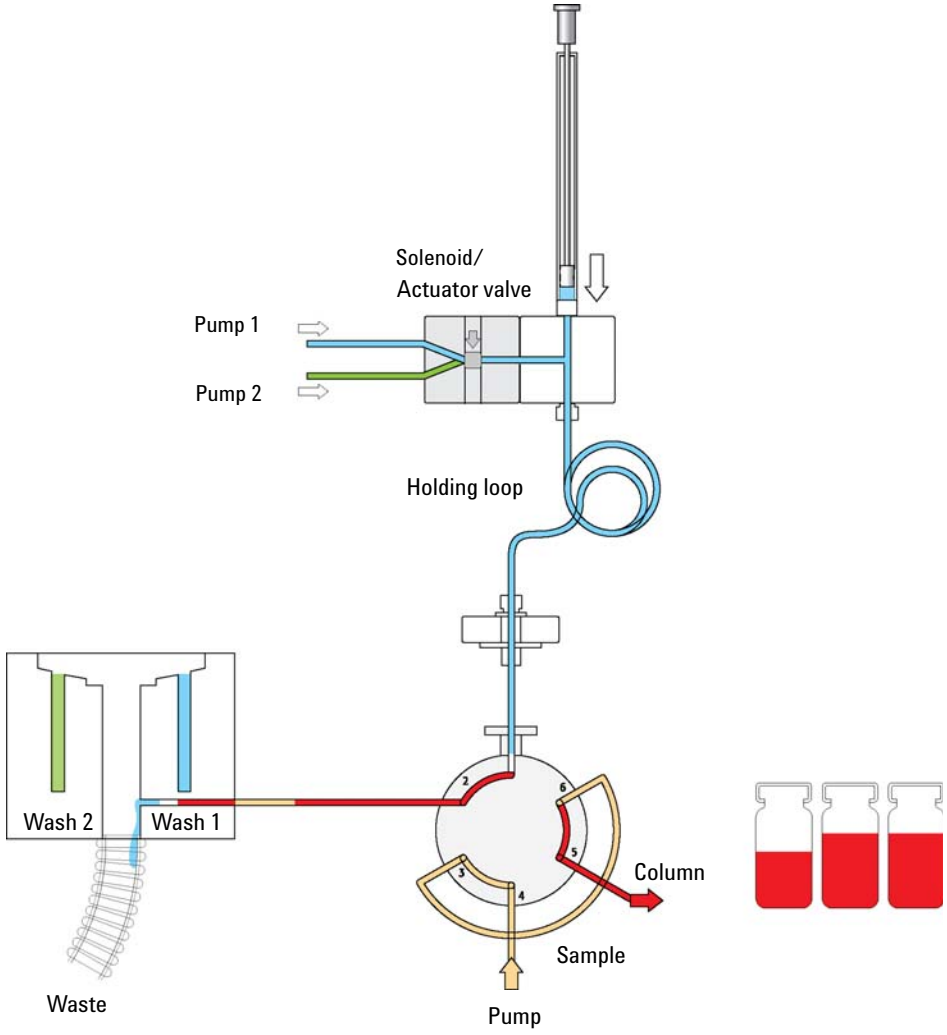


Figure 85 Standard Injection Cycle Step 12: Rear Sample Volume and Air Segment are dispensed to Waste

Standard: Step 13 — 14

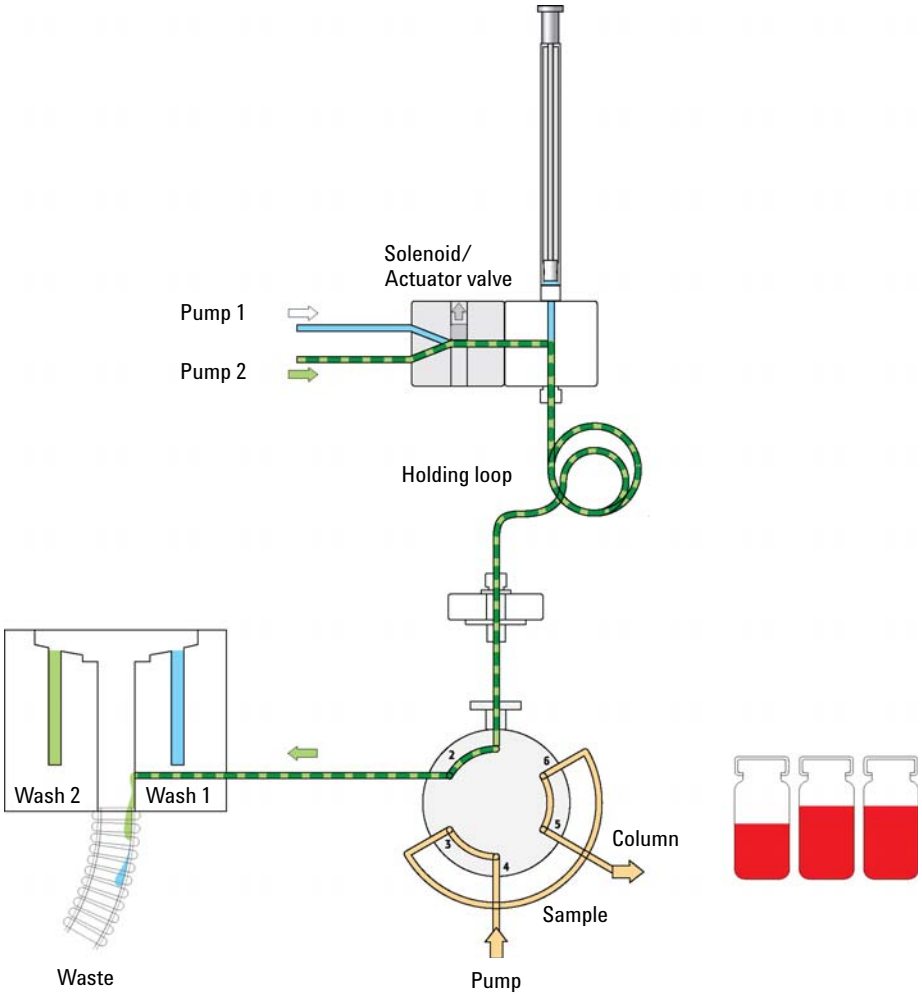


Figure 86 Standard Injection Cycle Step 13-14: Valve Clean with Wash Solvent 2

Standard: Step 15 — 16

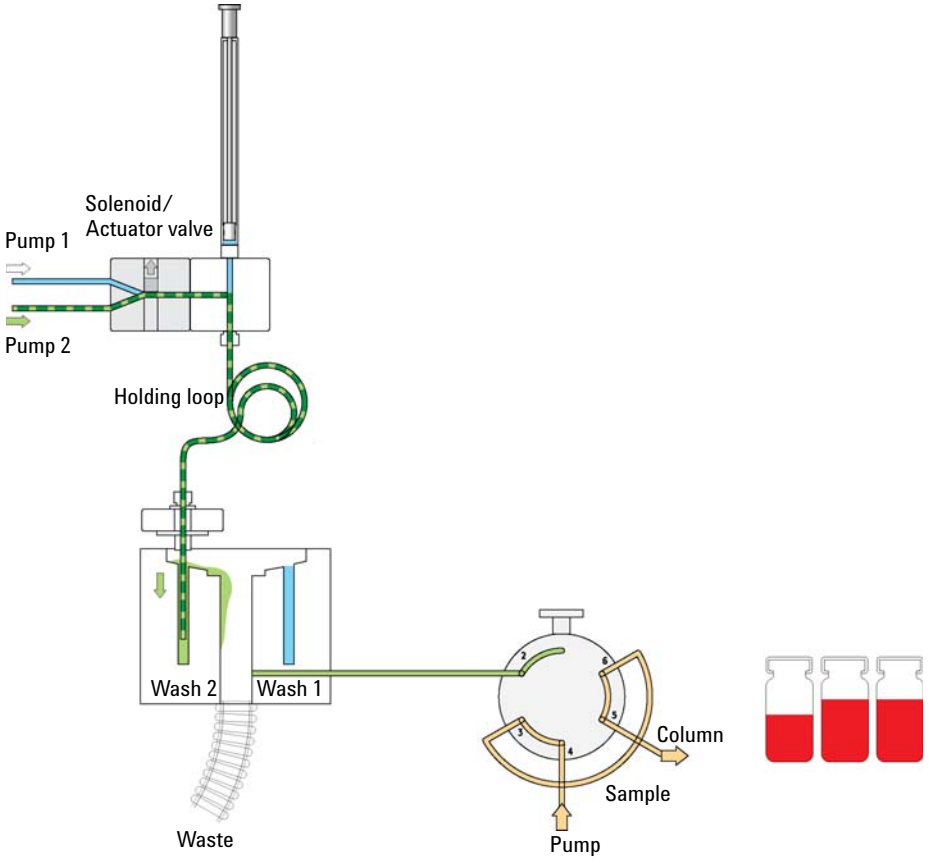


Figure 87 Standard Injection Cycle Step 15-16: Active Syringe Needle wash with Wash Solvent 2

Standard: Step 17 — 18

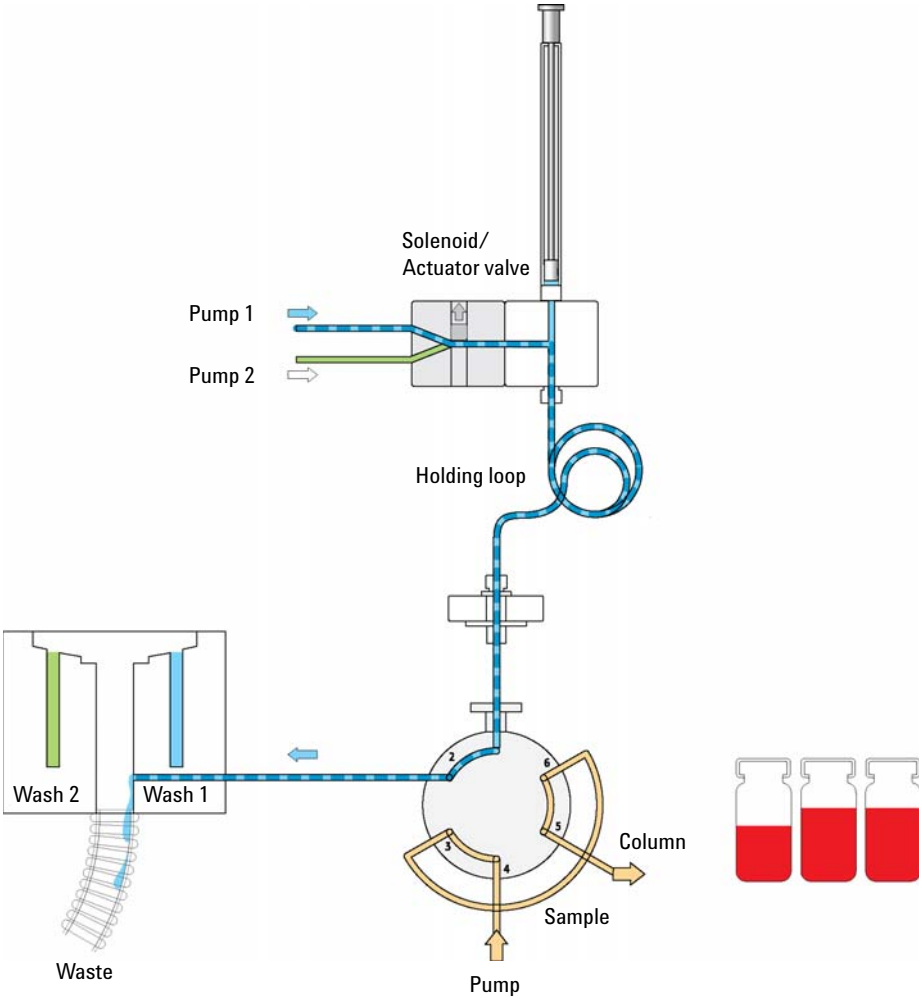


Figure 88 Standard Injection Cycle Step 17-18: Valve Clean with Wash Solvent 1

Standard: Step 19 — 20

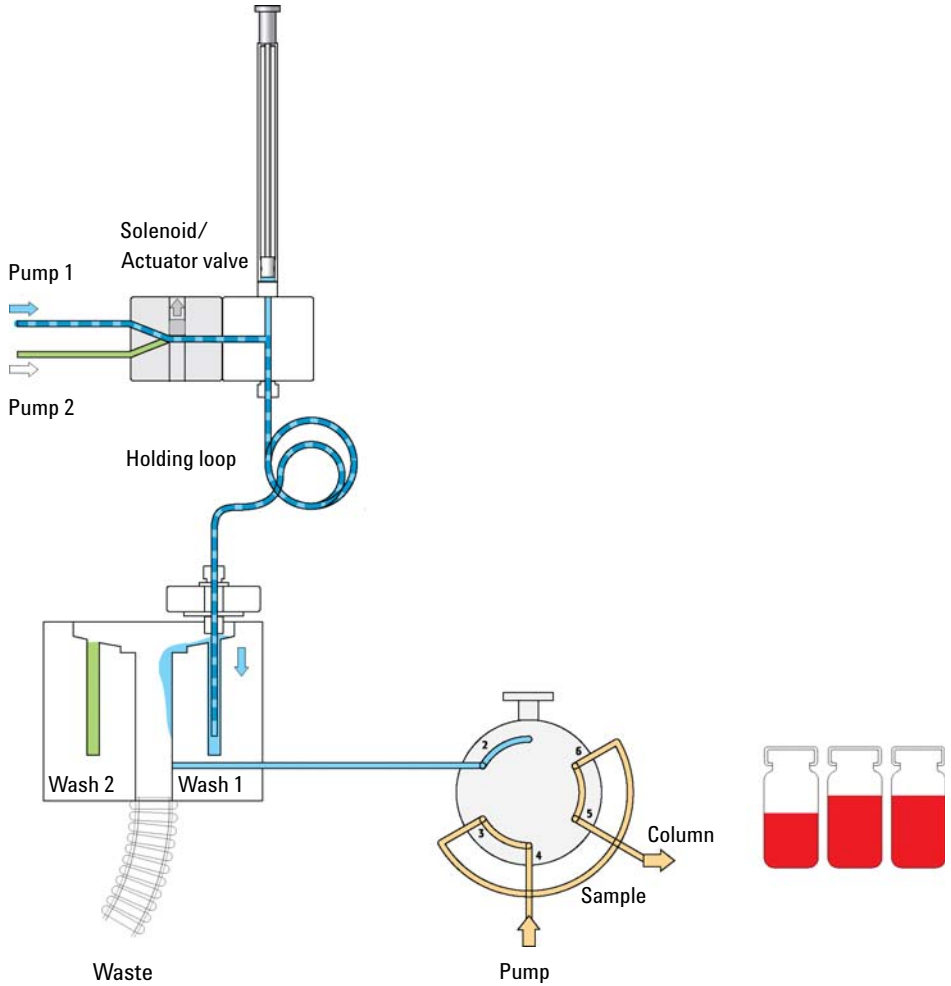


Figure 89 Standard Injection Cycle Step 19-20: Active Syringe Needle Wash with Wash Solvent 1

Standard: Cycle End

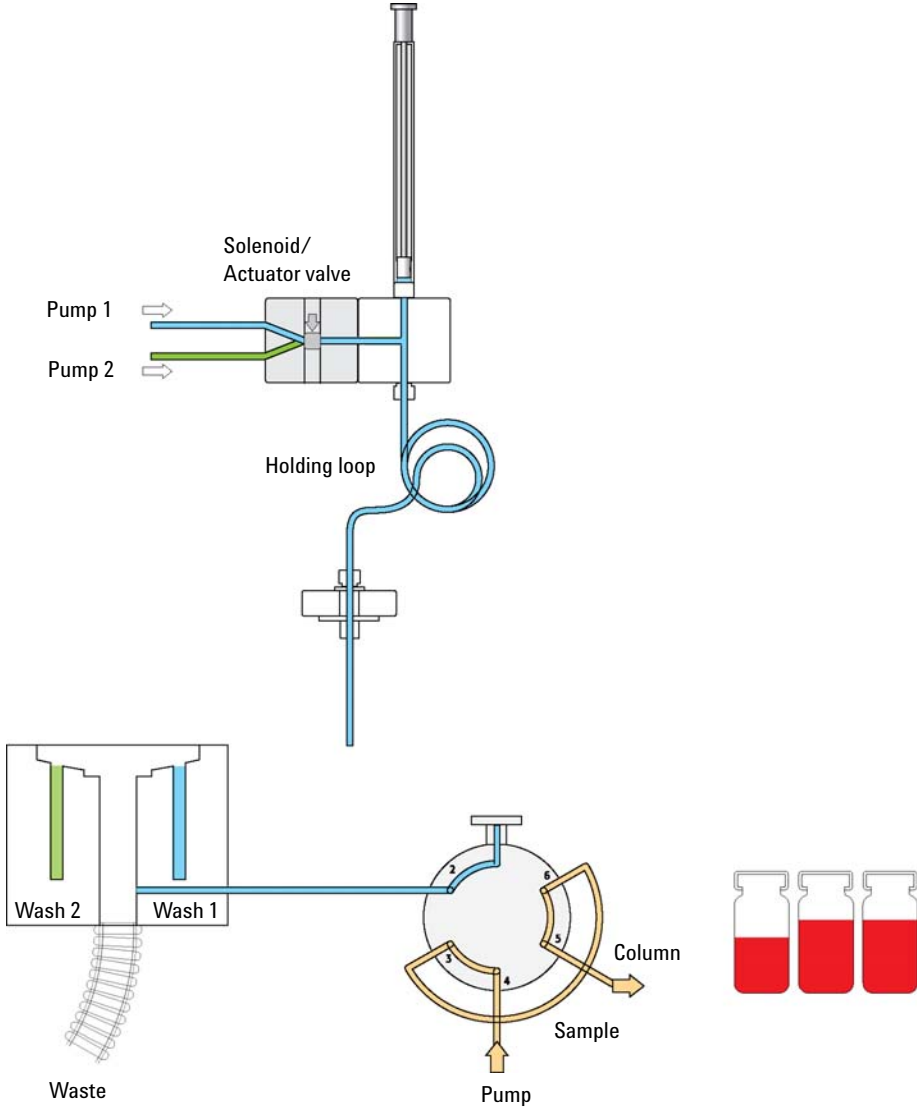


Figure 90 Standard Injection Cycle End

Additional Valve Toggle Step to the DLW Standard Cycle

Considerations for additional 'Stator Wash' Cleaning Step

The DLW Standard Cycle has the built-in option for the user to toggle the injection valve at the end of the chromatographic run before equilibration of the column to the start conditions. If the method variable ' **Stator Wash**' is set to ' **1**', the extra cleaning process for the valve, with ' **Valve Toggle**', becomes part of the standard cycle.

If the Method variable is deactivated (setting ' **0**'), the DLW Standard cycle ends as shown in Figure 90 on page 225

The macro (cycle) is written such that the optional valve toggle steps can be executed before re-equilibration of the column. The exact time the valve needs to be switched on hast to be synchronized with the chromatographic method by the user, Method variable ' **Delay Stator Wash**'. The two wash solvents are timed by the Method variables ' **Stator Wash Time Solvent 1**' and ' **Stator Wash Time Solvent 2**'. After these wash times have elapsed, the valve is switched back to the start position.

Figure 91 on page 226illustrates the recommended retention time for ' **Stator Wash**' or ' **Valve Toggle**' times.

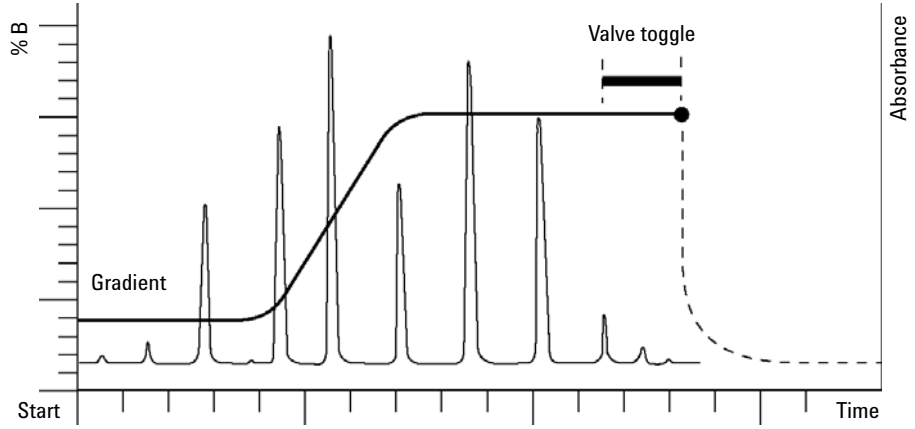


Figure 91 Timing for "**Stator Wash**" Step

From the chromatographic point of view, it is important to understand the optional cleaning step in detail. It is assumed that the valve stator between

ports 1 and 6 (example standard Cheminert Valve) is contaminated and cannot be cleaned during the standard injection process. The valve toggle brings the engraving back between the two ports. Flushing the valve with both wash solvents eliminates any remaining sample material located between stator ports 1 and 6.

What points need to be considered when using the ' **Stator Wash**'or ' **Valve Toggle**' option?

- Observe the rules if biofluid samples are injected. The first sample contact should always be with an aqueous solution to avoid protein precipitation. After washing with an organic solvent (higher elution power) the system must be flushed again with Wash Solvent 1.
- The first toggle near the end of the chromatographic cycle provides the advantage that the sample loop is already flushed out, first using the mobile phase with a solvent of high elution power (assuming gradient application).
- The second valve toggle time follows immediately after the second solvent flush is completed. A second switching time cannot be programmed. The waiting period before the second valve toggle should be long enough so that the entire system is flushed out by both wash solvents.

  Consider the entire delay volume to determine the second valve toggle time. The DLW internal volumes are:

  - Manifold, 90 µl,
  - Holding Loop, 118 µl(inclusive Needle DLW-2 version,
  - Installed Injection Loop.

  Total delay volume: 208 µl+ Loop content volume.
- The second valve toggle (back to starting conditions) should be done before the system equilibration time has started. The Loop contents should ideally be a solvent of a low elution power when switched back.
- If isocratic chromatography is applied, it is possible that the remaining contaminants are washed into the system and can build up higher background noise for the column and/or detector over a longer period of time.

Additional Cleaning step 'Stator Wash' or 'Valve Toggle' Step-by-Step

Stator Wash: End of Standard Injection Cycle, Start for additional Cleaning Step 'Valve Toggle'

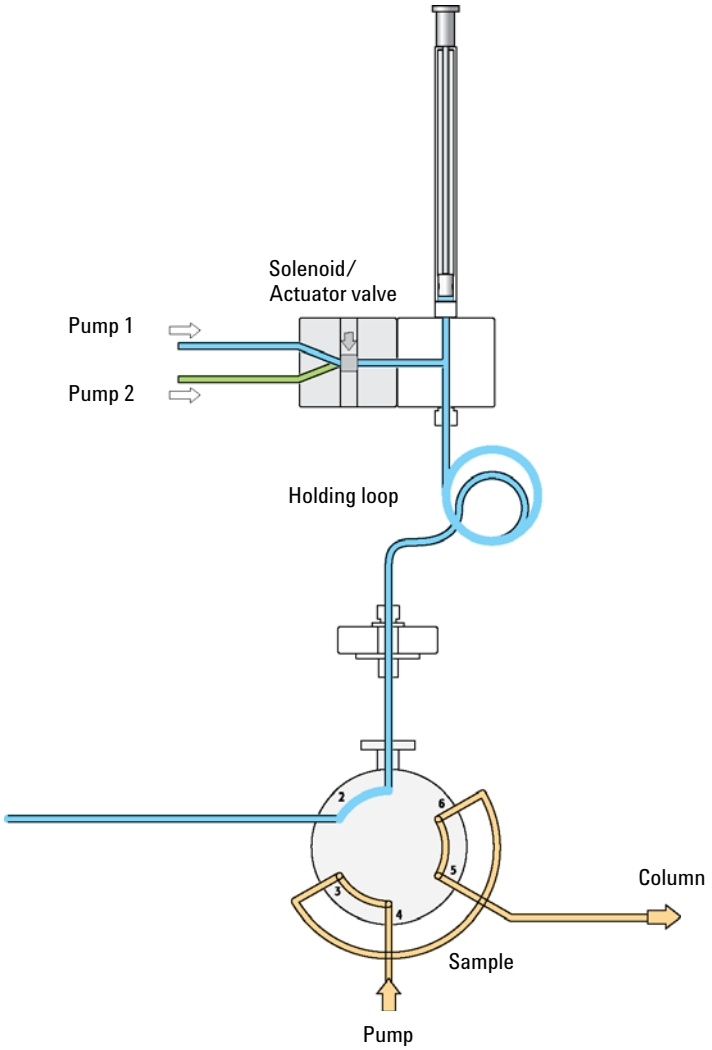


Figure 92 Additional Cleaning Step: End of Standard Injection Cycle, Start for additional Cleaning Step '**Valve Toggle**'

Stator Wash: Steps 1 – 2

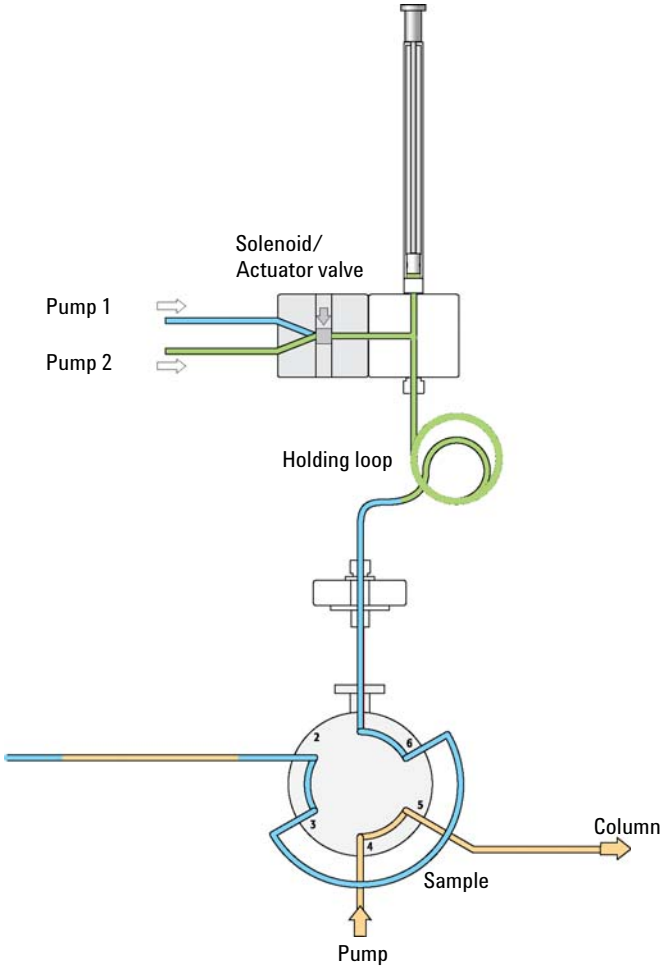


Figure 93 Additional Cleaning Step: Valve Switched to Load Position (toggle), Valve clean with Wash Solvent 1

Stator Wash: Step 3

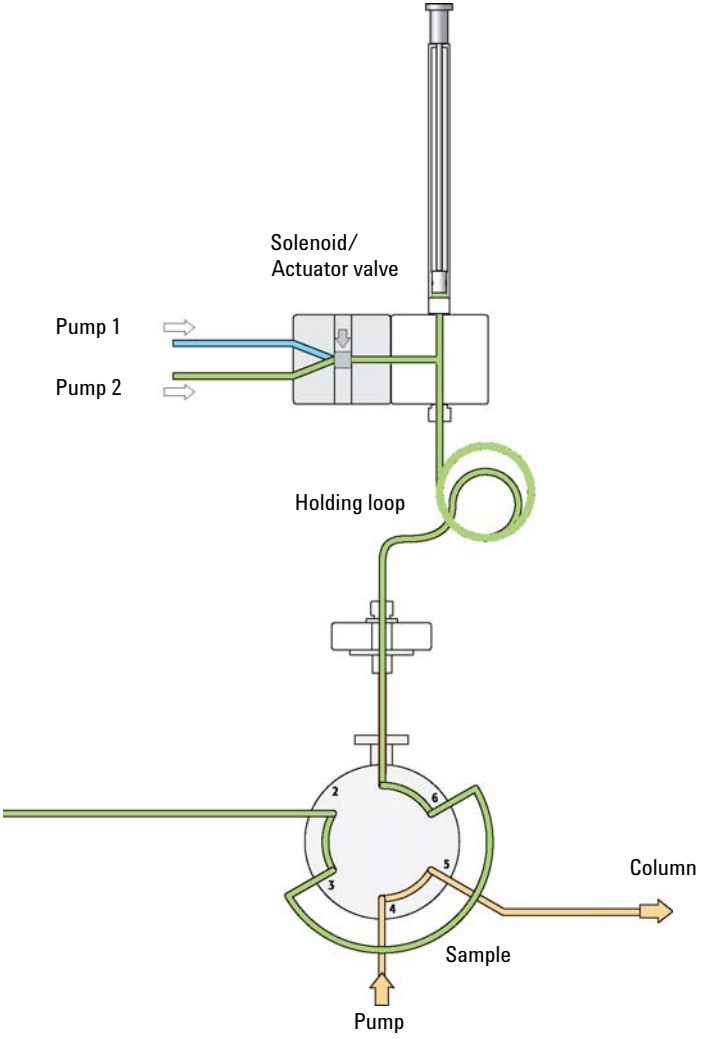


Figure 94 Additional Cleaning Step: Valve Clean with Wash Solvent 2

Stator Wash: Step 4

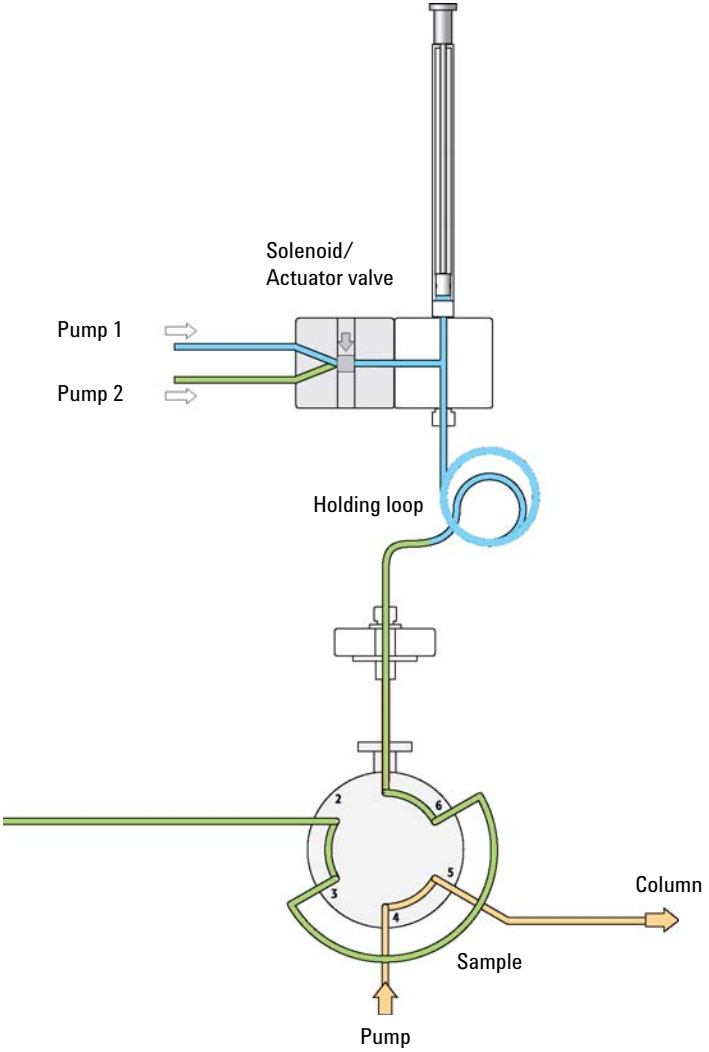


Figure 95 Additional Cleaning Step: Wash Solvent 2 dispensed by Wash Solvent 1

Stator Wash: Step 5

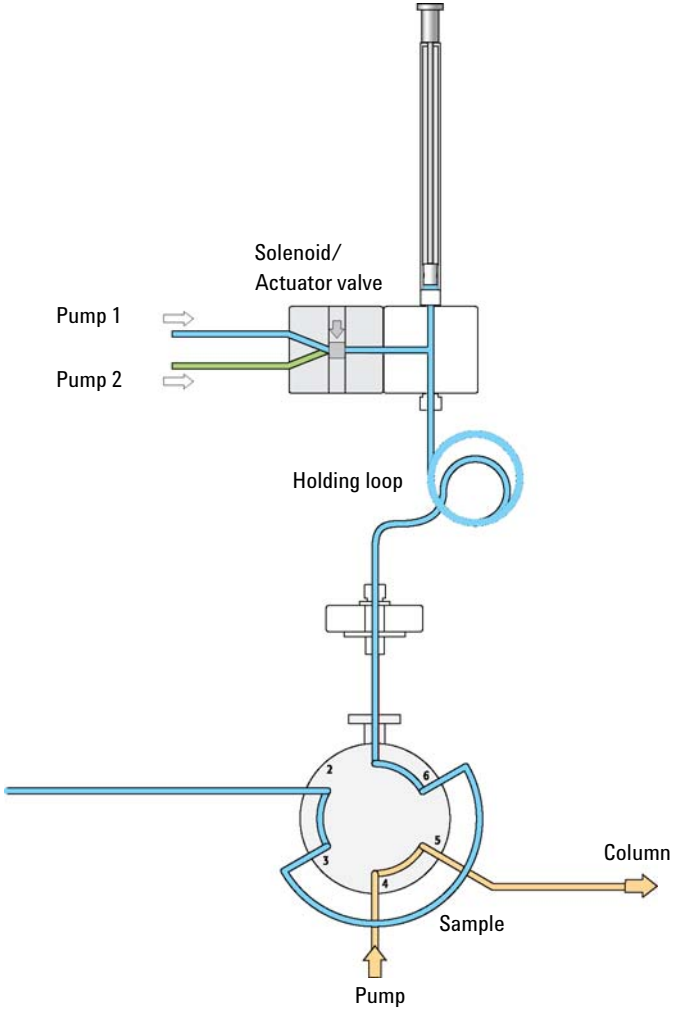


Figure 96 Additional Cleaning Step: Second Valve Clean with Wash Solvent 1

Stator Wash: Step 6

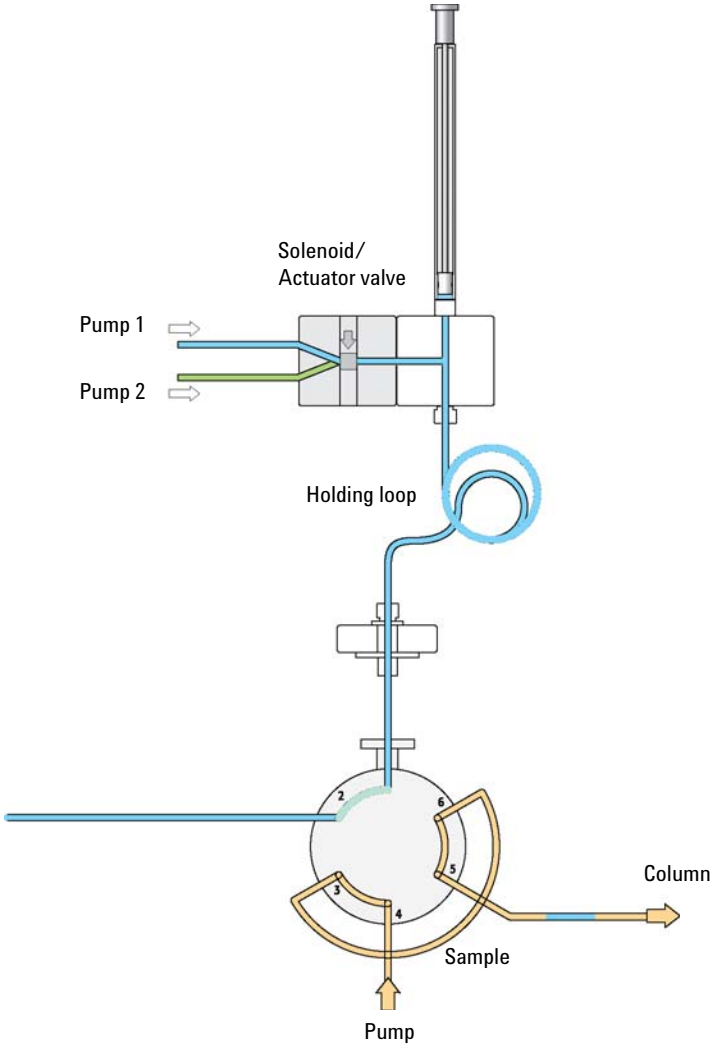


Figure 97 Additional Cleaning Step: Valve switched back to Inject Position (toggle)

Fast Injection Cycle Step-by-Step

Fast: Cycle Start

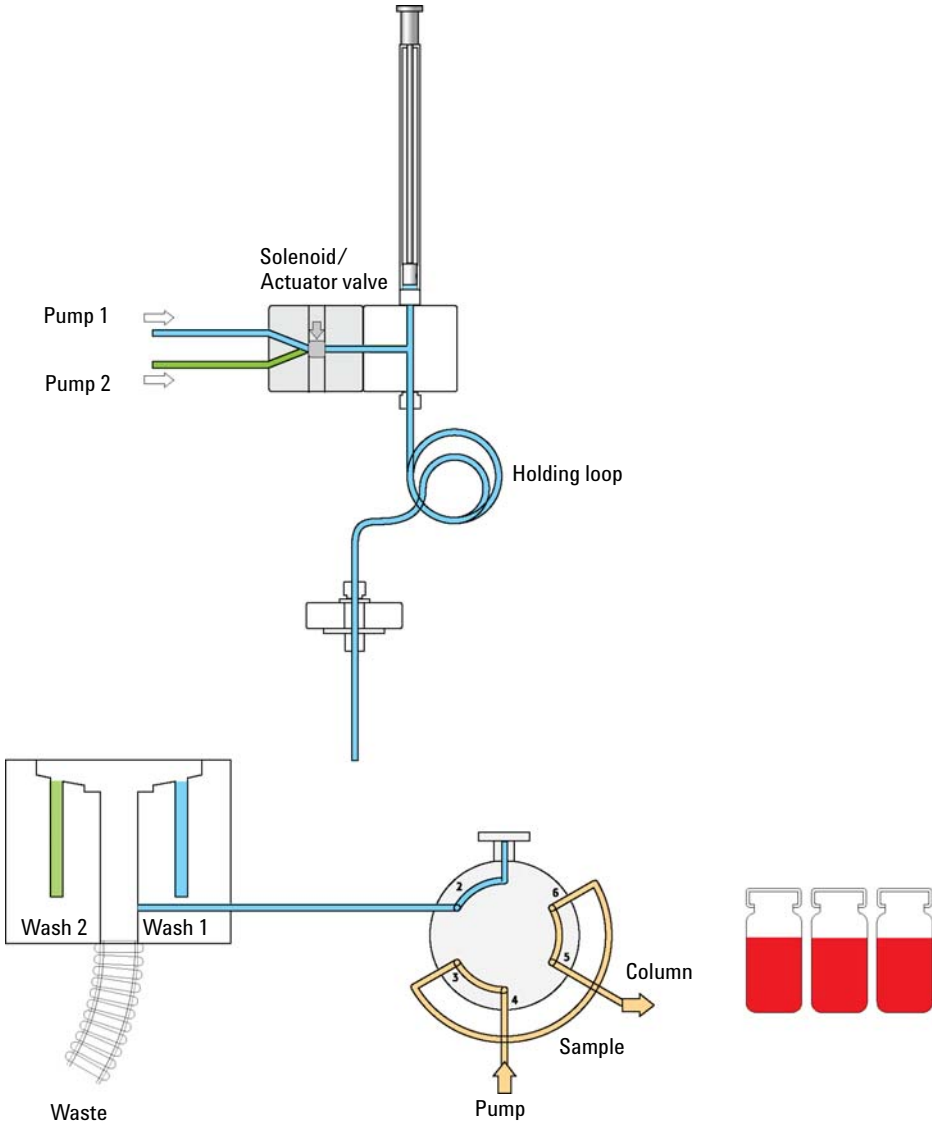


Figure 98 Fast Injection Cycle: Start

Fast: Step 1

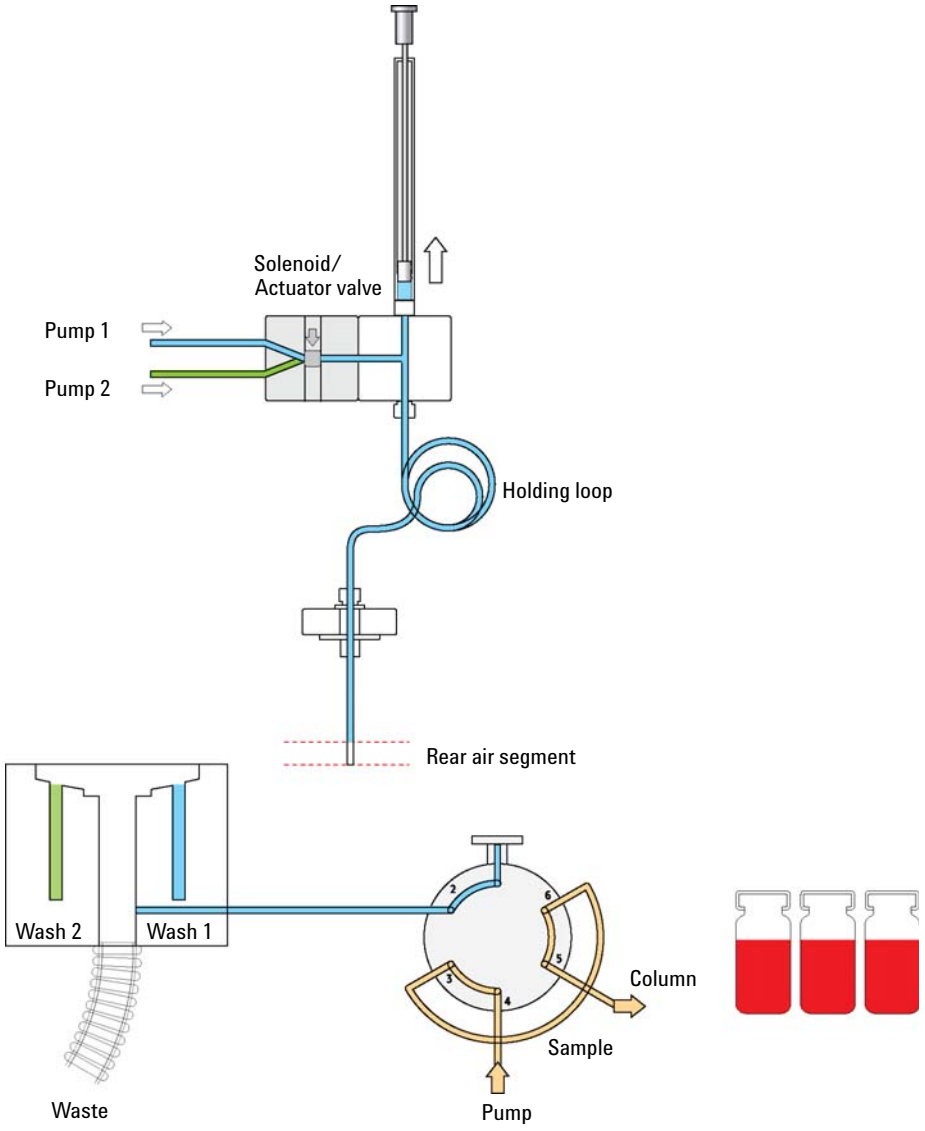


Figure 99 Fast Injection Cycle: Aspirate Rear Air Segment

Fast: Step 2

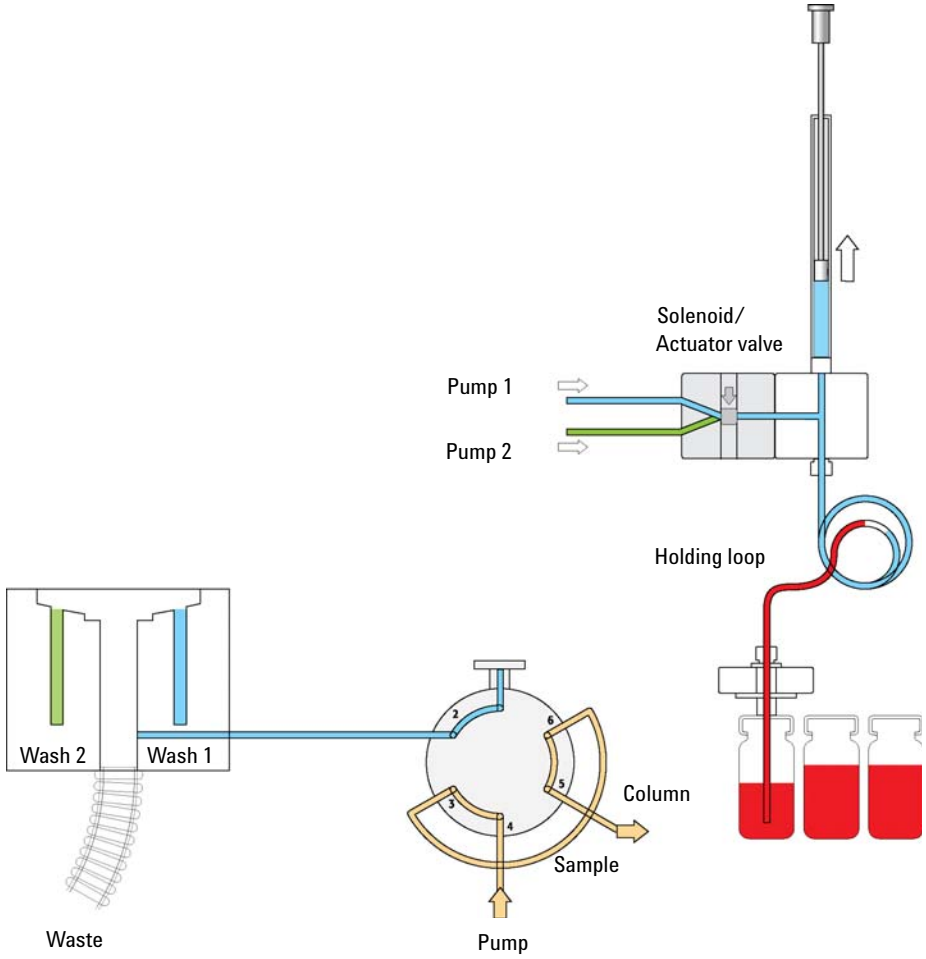


Figure 100 Fast Injection Cycle: Get Sample, Aspirate Rear, Inject and Front Volume

Fast: Steps 3-4

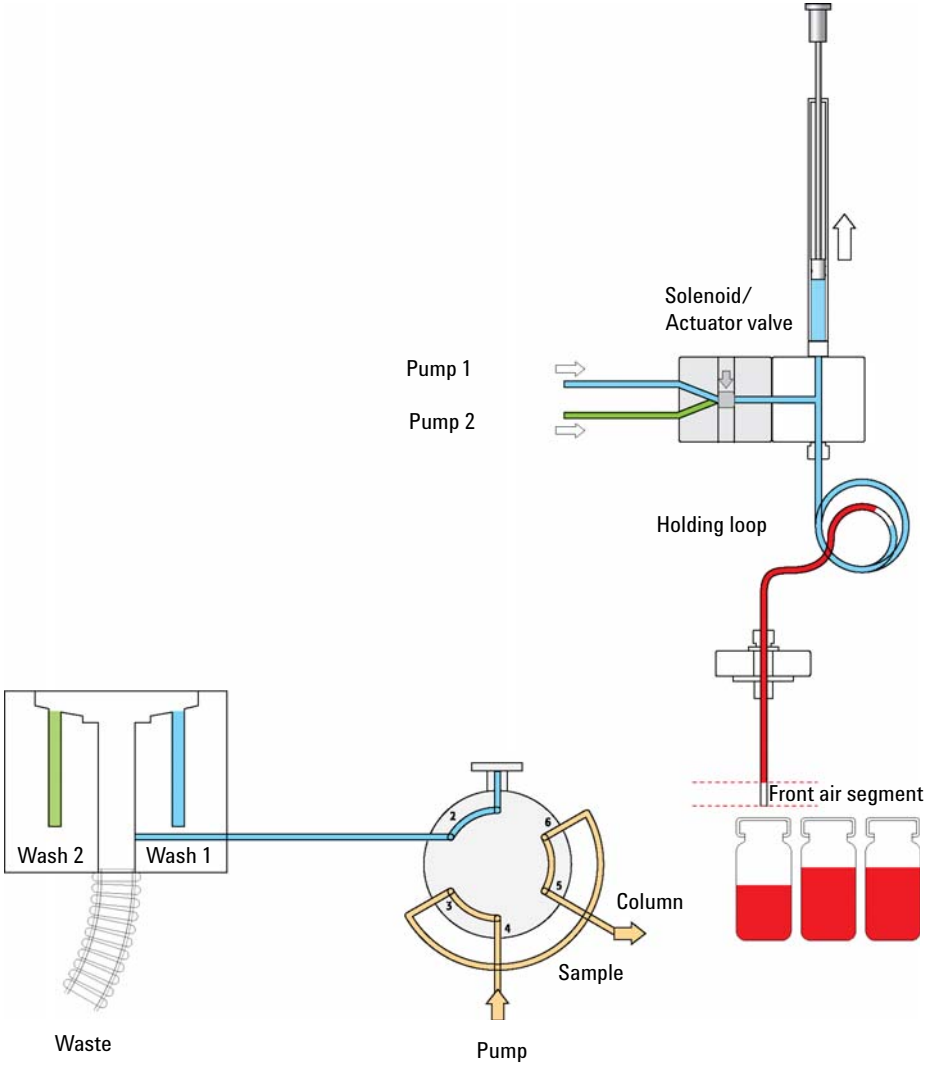


Figure 101 Fast Injection Cycle: Aspirate Front Air Segment

Fast: Steps 5 - 6

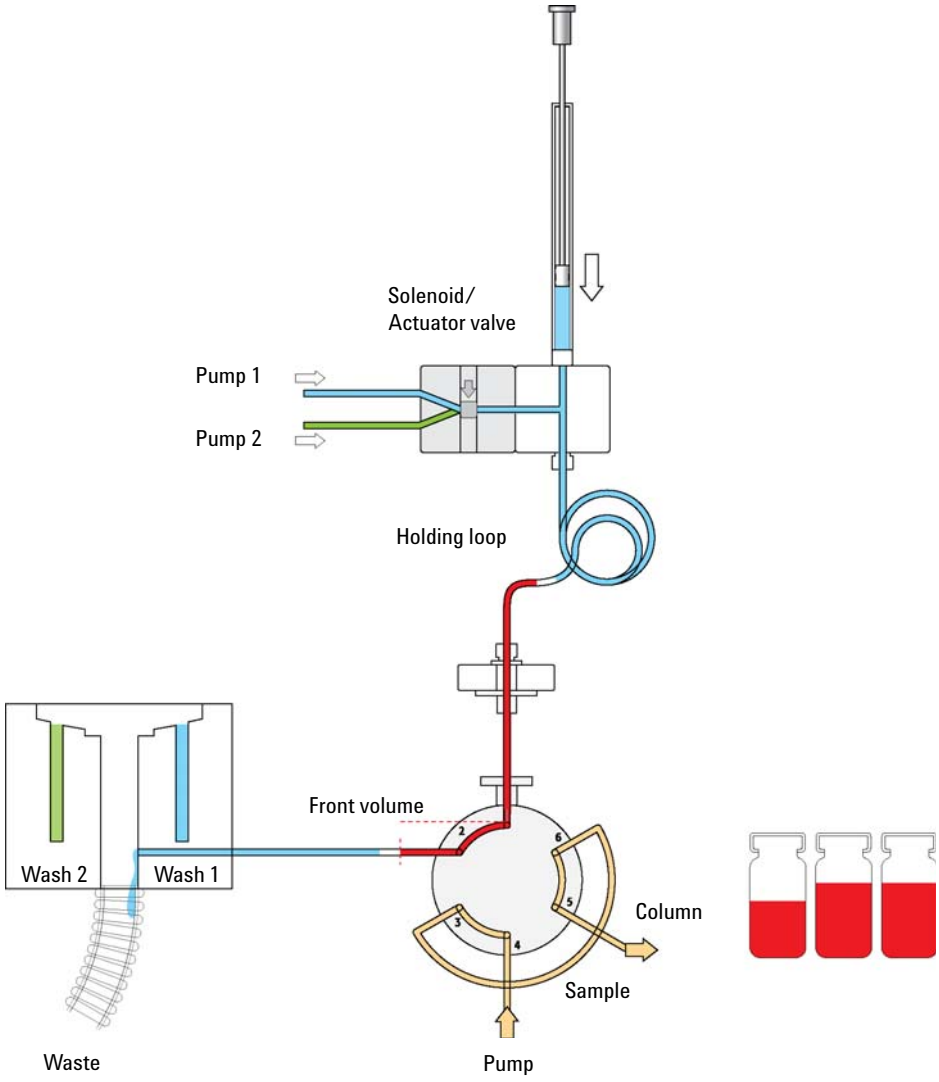


Figure 102 Fast Injection Cycle: Dispense Font Air Segment and Front Sample Volume to Waste

Fast: Steps 7 - 8

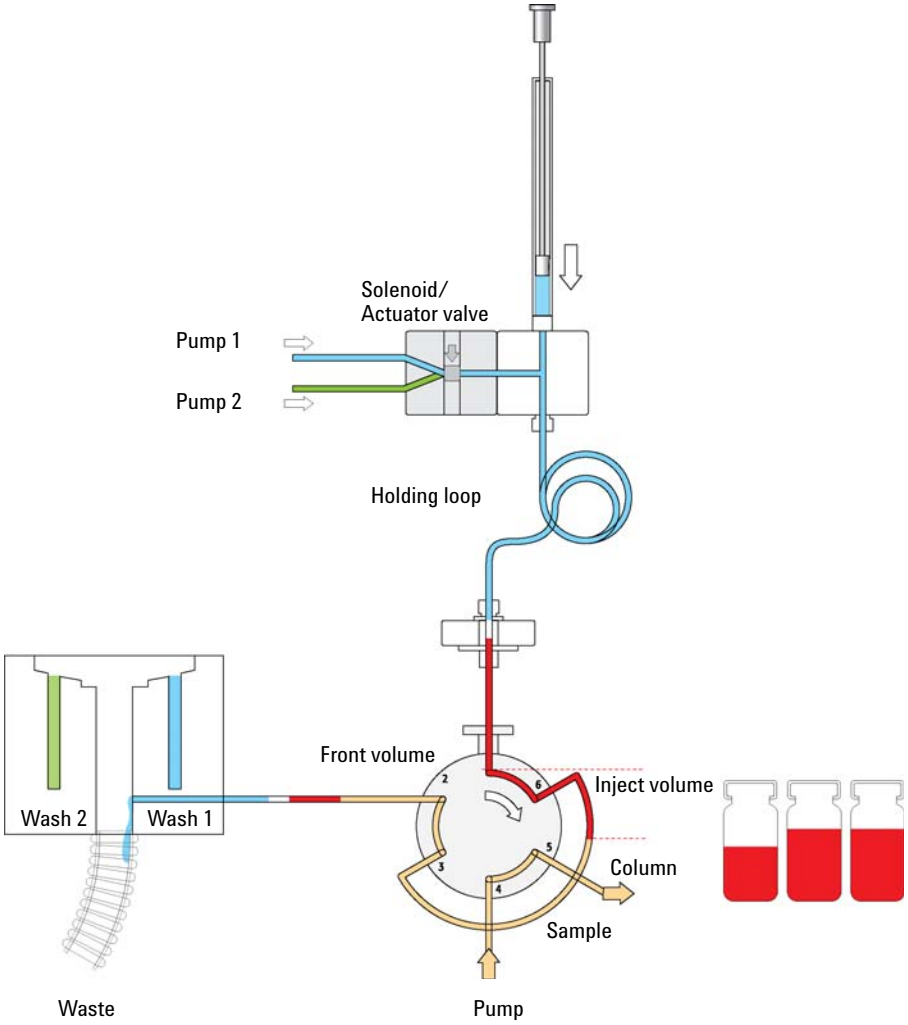


Figure 103 Fast Injection Cycle: Valve is switched to LOAD position, Loop is filled with '**Inject Volume**'

Fast: Step 9

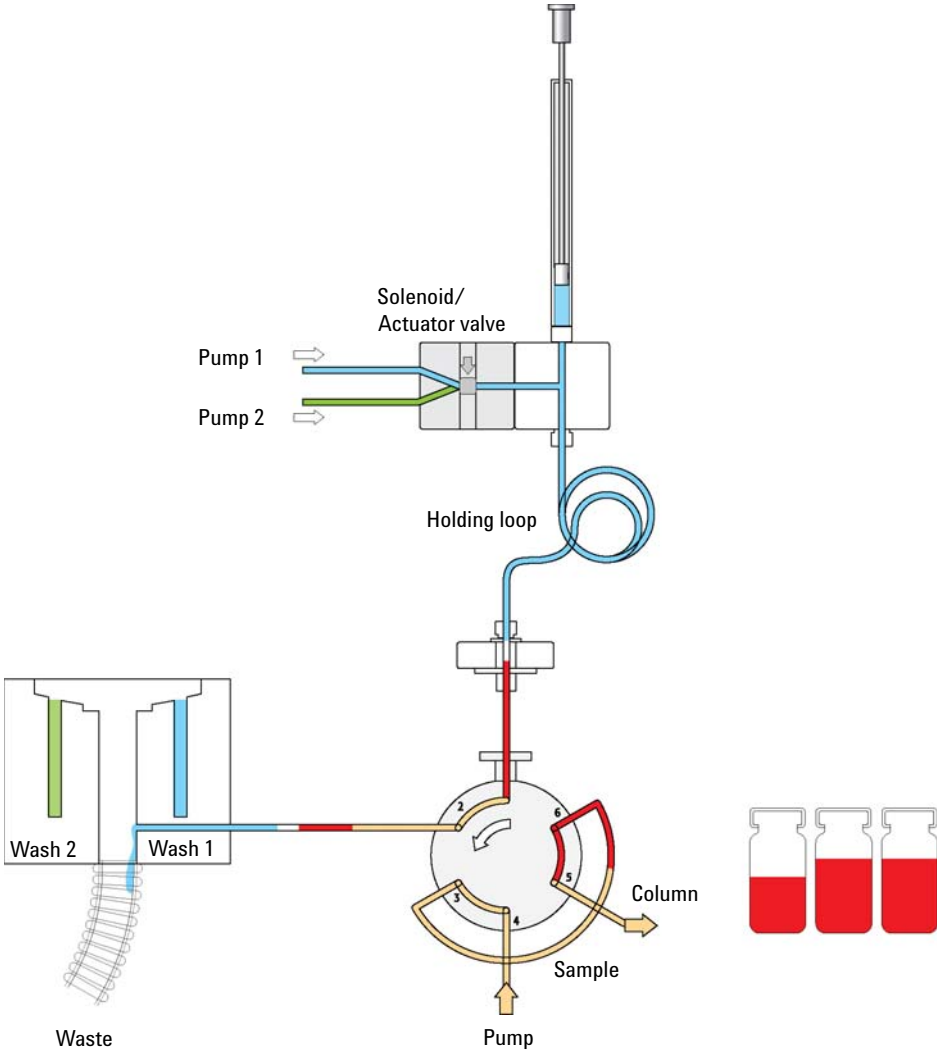


Figure 104 Fast Injection Cycle: Valve is switched to INJECT position, start chromatographic process

Fast: Step 10

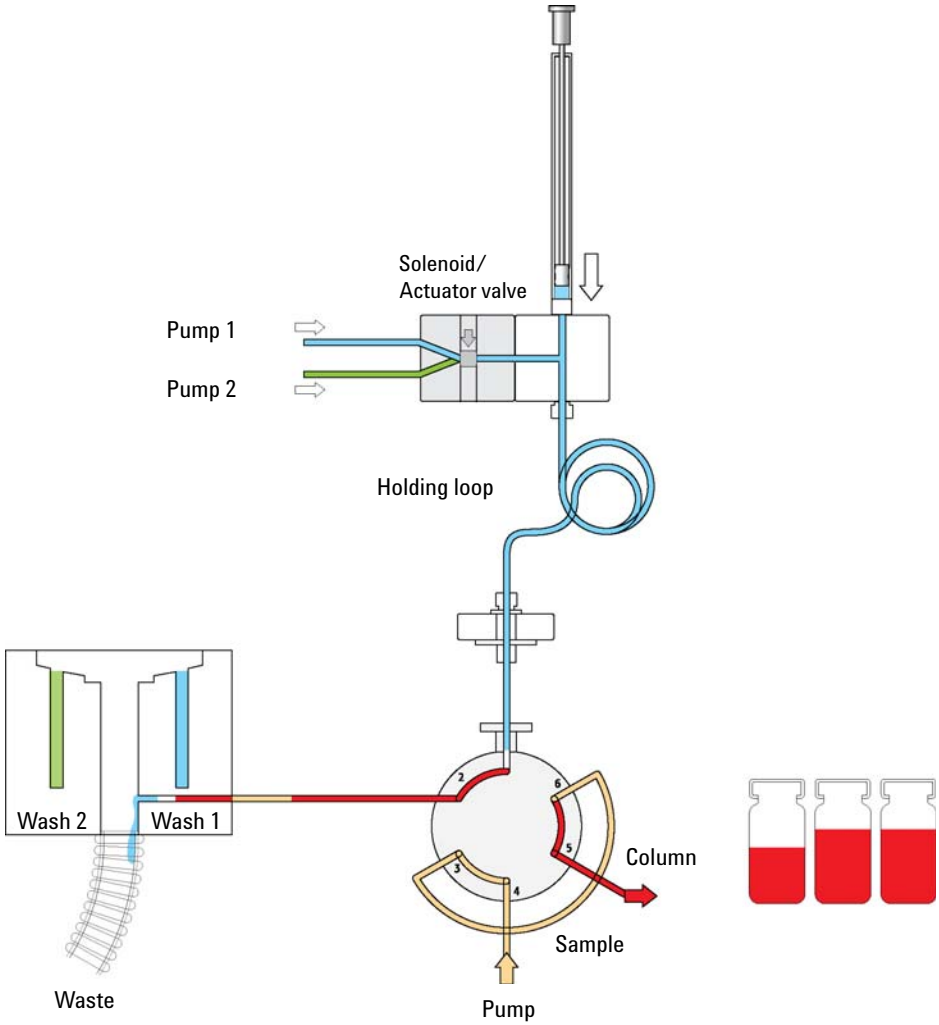


Figure 105 Fast Injection Cycle: Rear Sample Volume and Air Segment are dispensed to Waste

Fast: Steps 11 - 12

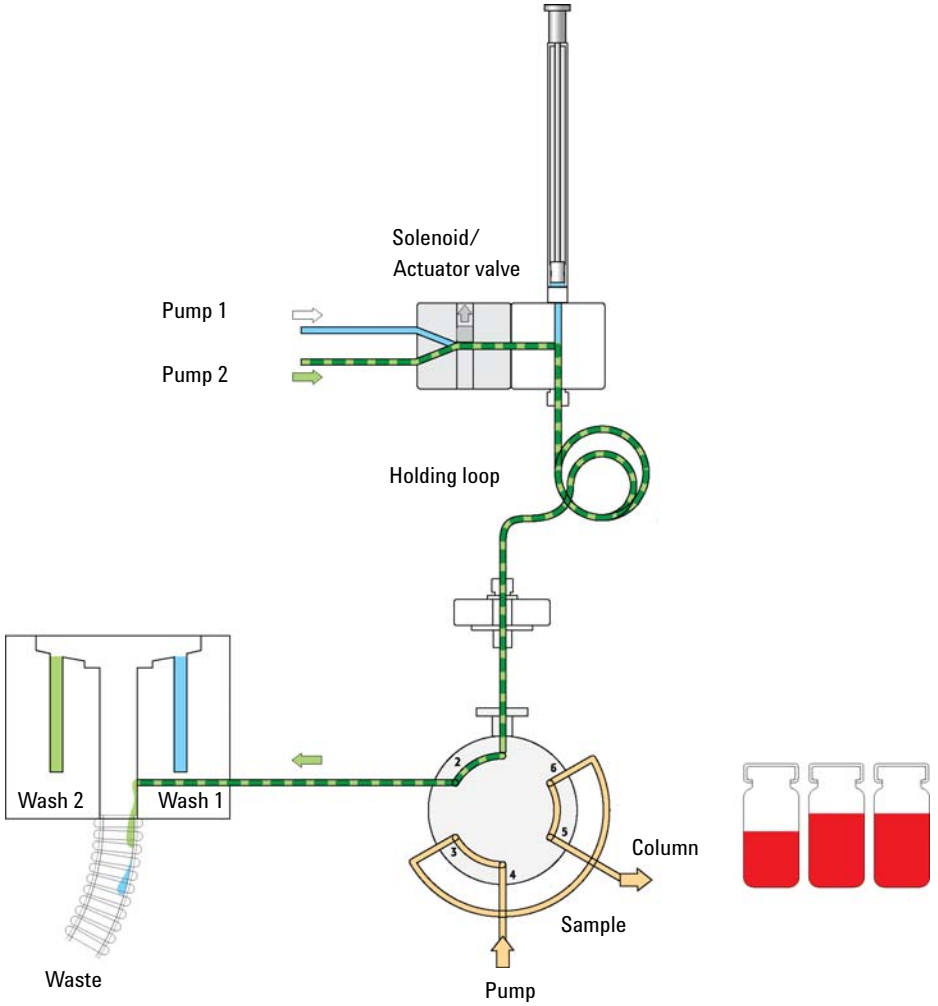


Figure 106 Fast Injection Cycle: Valve Clean with Wash Solvent 2

Fast: Steps 13 - 14

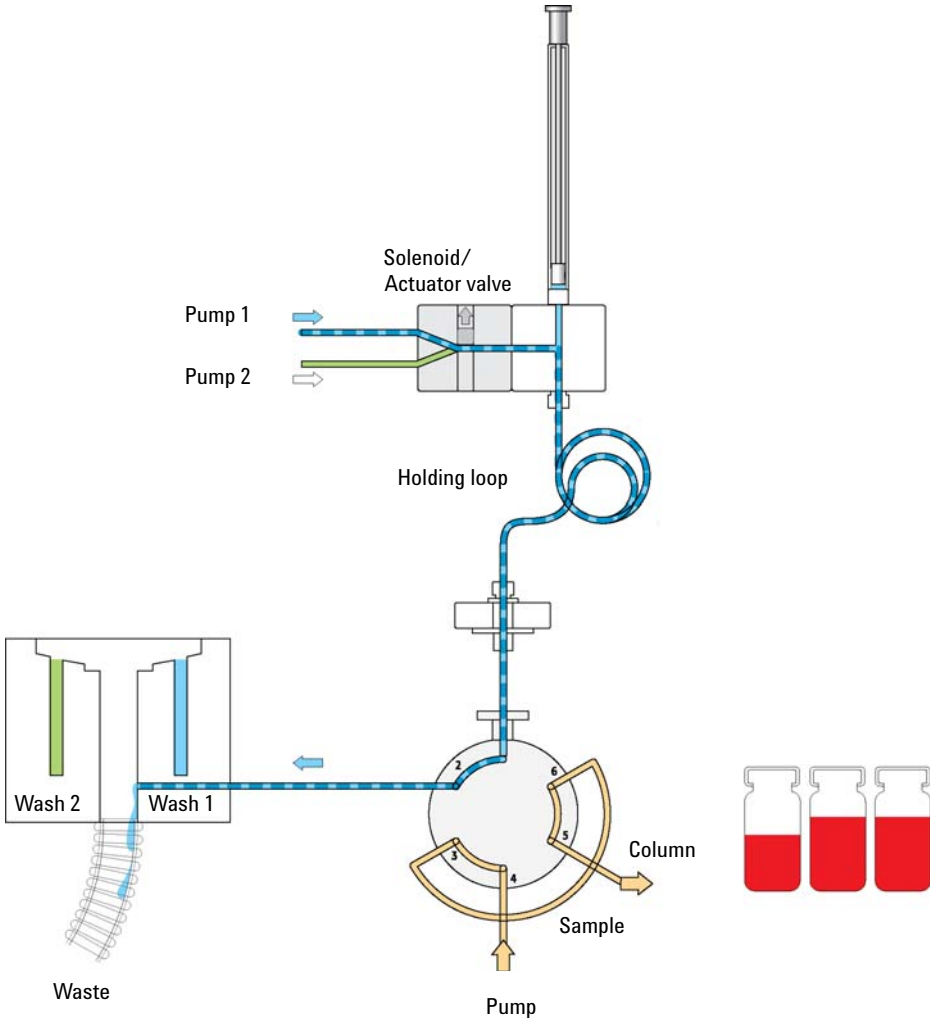


Figure 107 Fast Injection Cycle: Valve Clean with Wash Solvent 1

Fast: Step 15

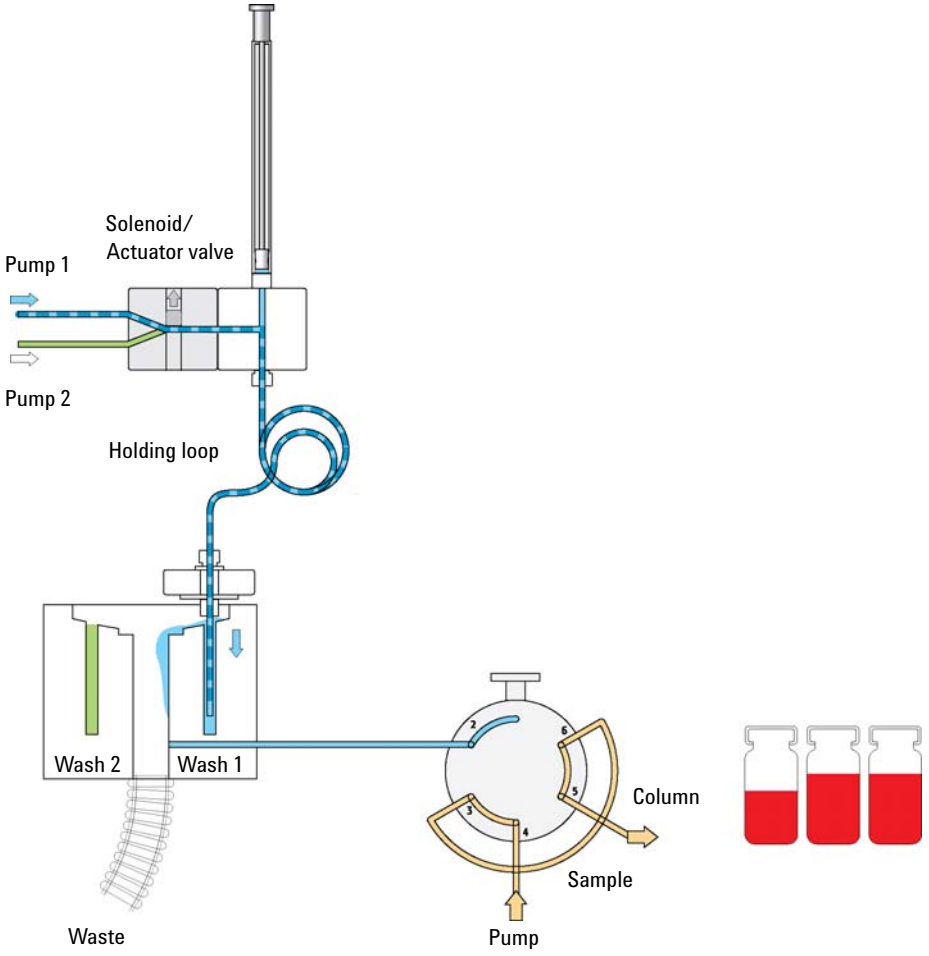


Figure 108 Fast Injection Cycle: Active Syringe Needle Wash with Wash Solvent 1

Fast: Cycle End

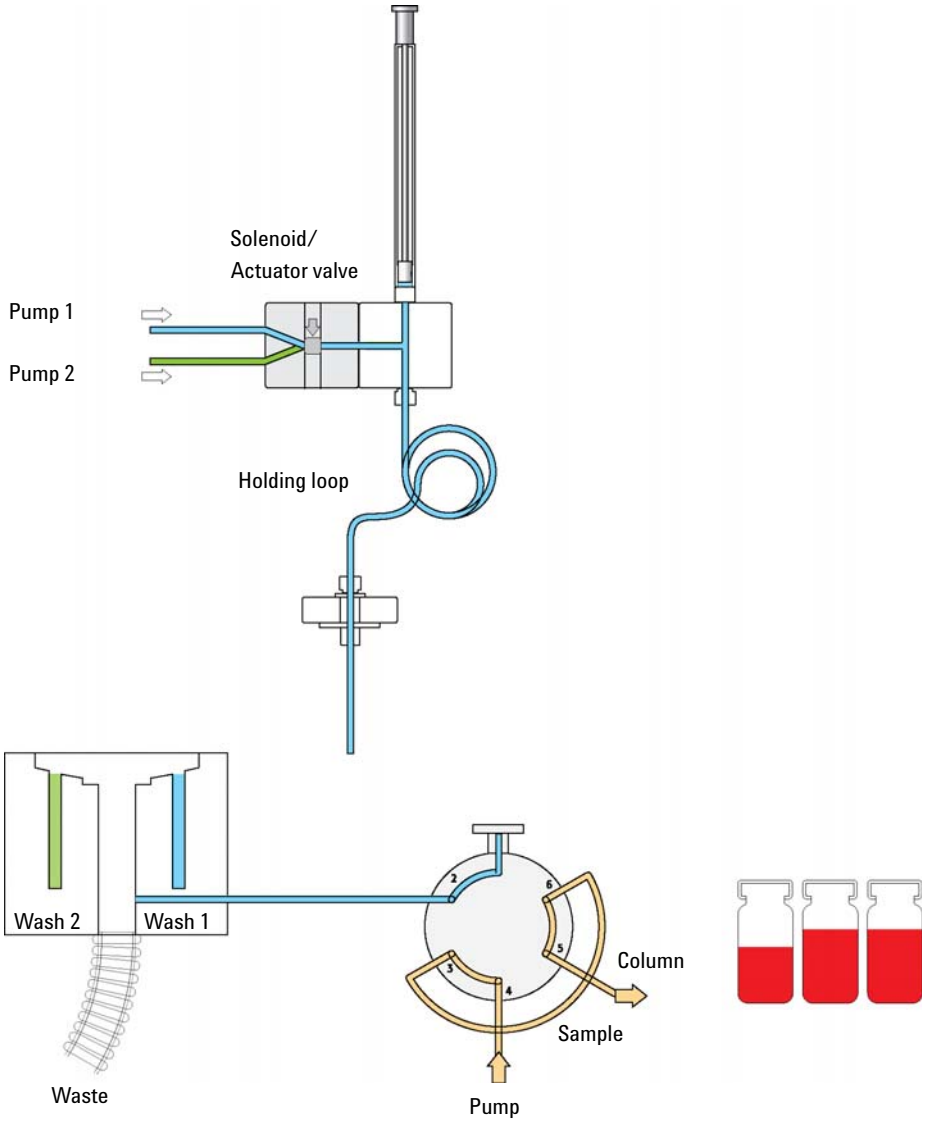


Figure 109 Fast Injection Cycle: End

Software Flow Chart

LC Injector HTC/HTS-xt System Flow Chart based on PAL Firmware version 4.1.X (HTC)

NOTE Firmware version 4.1.X., as shown in the flow chart, corresponds to the LC Injector HTC/HTS-xt System. The changes from Firmware version 2.5.X., as used for the LC Injector HTC/HTS System, are emphasized in this LC Injector HTC/HTS User Manual.

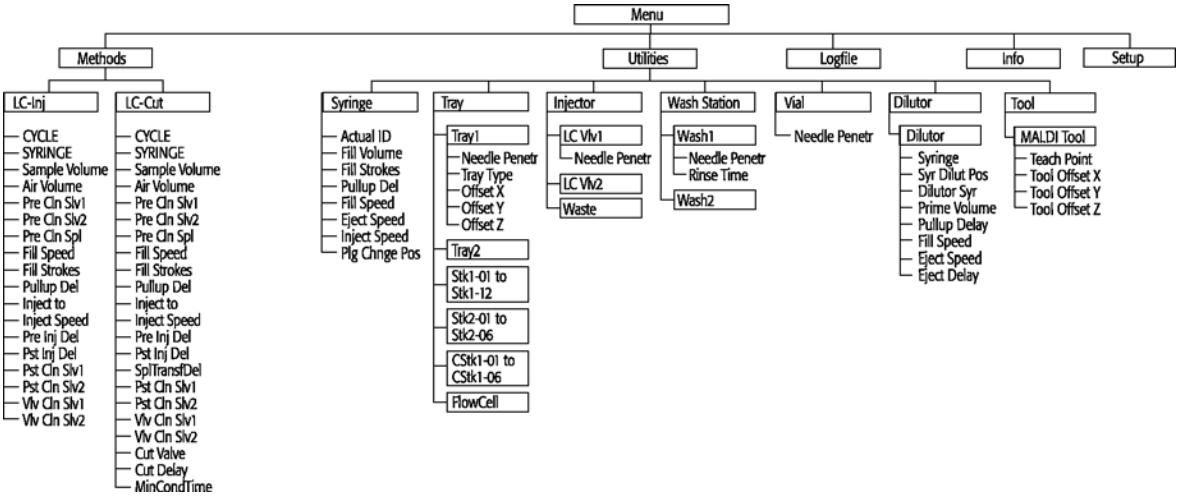


Figure 110 LC Injector HTC-xt Firmware Overview, Page 1

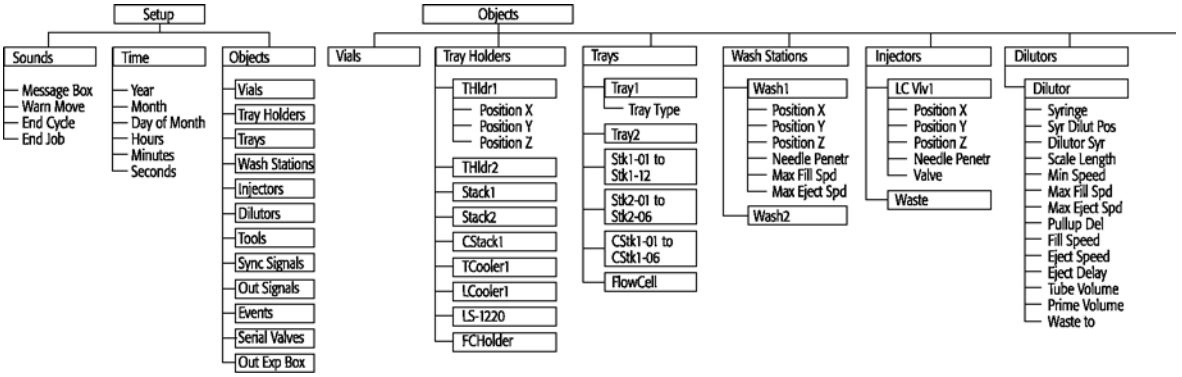


Figure 111 LC Injector HTC-xt Firmware Overview, Page 2

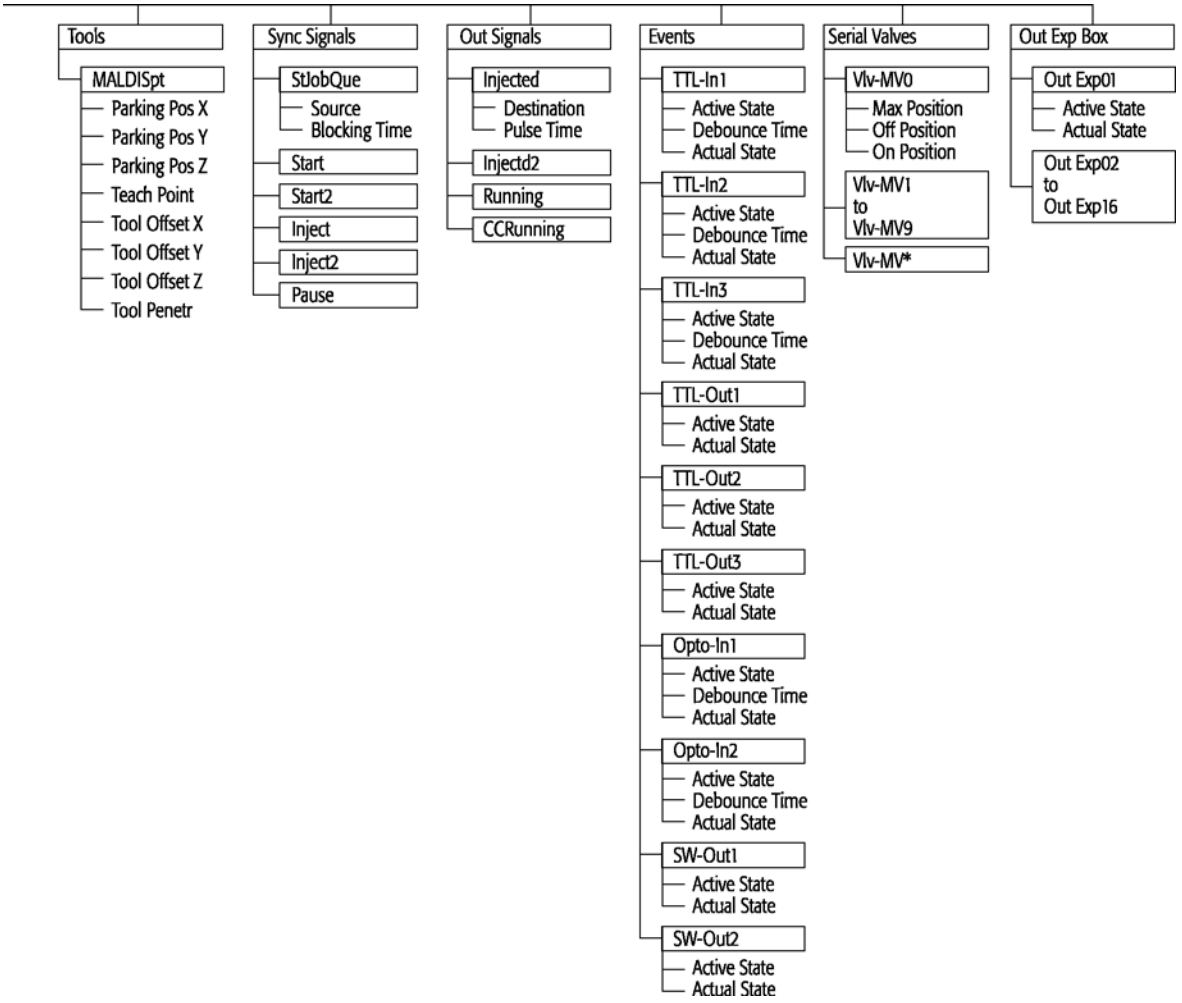


Figure 112 LC Injector HTC-xt Firmware Overview, Page 3

Method Cycle	Recommended PAL Method Parameters
LC-Inj	
CYCLE	LC - Inj
SYRINGE	100 µl
Sample Volume	80 µl
Air Volume	0 nl
Pre Cln Slv1	1
Pre Cln Slv2	0
Pre Cln Spl	2
Fill Speed	10 µl/s
Fill Strokes	3
Pullup Del	3-10 s
Inject to	LC Vlv 1
Inject Speed	10 µl/s
Pre Inj Del	500 ms
Pst Inj Del	500 ms
Pst Cln Slv1	2
Pst Cln Slv2	0
Vlv Cln Slv1	2
Vlv Cln Slv2	0

Method Cycle	Recommended PAL Method Parameters	Remarks
LC-Cut		
CYCLE	LC - Cut	
SYRINGE	100 µl	
Sample Volume	80 µl	
Air Volume	0 nl	
Pre Cln Slv1	1	
Pre Cln Slv2	0	
Pre Cln Spl	2	
Fill Speed	5 µl/s	
Fill Strokes	3	
Pullup Del	3-10 s	
Inject to	LC Vlv 1	
Inject Speed	5 µl/s	
Pre Inj Del	500 ms	
Pst Inj Del	500 ms	
SplTransfDel	Sample Transfer Delay	Time needed to transfer sample from Loop onto 2nd valve (pre - or analytical column)
Pst Cln Slv1	2	
Pst Cln Slv2	0	
Vlv Cln Slv1	2	
Vlv Cln Slv2	0	
Cut Valve	LC Vlv 2	Specify name of 2nd valve, switching valve
Cut Delay		Time needed for clean-up. Switch to backflush sample, to analytical column or detector
MinCondTime		Minimum Conditioning Time: Time needed to condition pre - column on 2nd valve

Example for the following conditions:

- Loop Size 20 µL, overfill 3 - 5 times
  Partial Loop filling: Allowed sample volume range 20 - 60% of loop content for loops ≤ 100 µL
  Larger Loops: 20 - 80% of loop content

- Syringe:
  Syr X G100 - 22S - 3: Gauge 22S limits fill speed to max 20 µL/s (5-20 µL/s depends on viscosity of solvent)
  Syr X G100 - 22 - 3: Gauge 22 allows fill speed up to 200 µL/s (depends on viscosity of solvent)

- Eject speed for 100 µL Syringe: 50 to 150 µL/s (Utilities/Syringe)

- Pre - and post Washing:
  Use Solvent 1 and 2 for samples with components of extremly different polarities.
  Samples containing proteins should not contact organic solvents.

- Wash Steps for biological samples:
  1st Wash Cycle: Aqueous solvent
  2nd Wash Cycle: Organic solvent

  1st Wash Cycle before next sample:
  Pre-wash with aqueous solvent (eliminate organic solvents in Syringe and Valve)

LC-Cut Cycle controls 2 valves.

LC Valve 1: injection valve with loop.
LC Valve 2: Switching valve.
- Example 1: Pre - column for sample clean-up, backflushing to analytical column. Cut Delay > 0.
- Example 2: 10 - port valve with 2 analytical columns. Loading column 1, condition column 2.
  Valve toggle after Cycle time. Loading column 2, condition column 1. Cut Delay = 0

Figure 113 LC Injector HTC-xt Firmware Overview, Page 4

PAL HTS-xt System Flow Chart based on PAL Firmware Level 4.1.X

NOTE Firmware version 4.1.X., as shown in the flow chart, corresponds to the PAL-xt System. The changes from Firmware version 2.5.X., as used for the PAL System, are emphasized in this PAL User Manual.

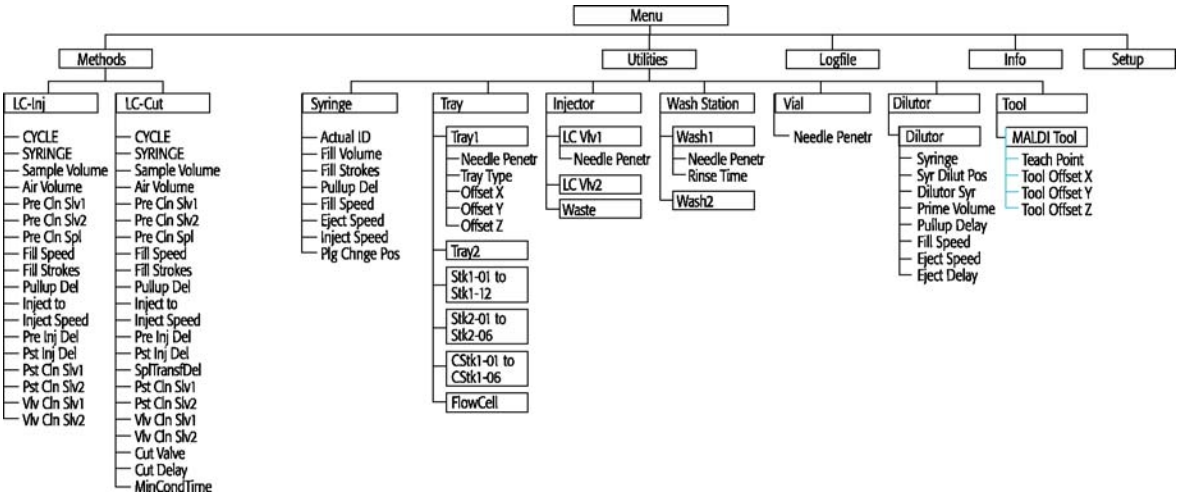


Figure 114 PAL HTS-xt Firmware Overview, Page 1

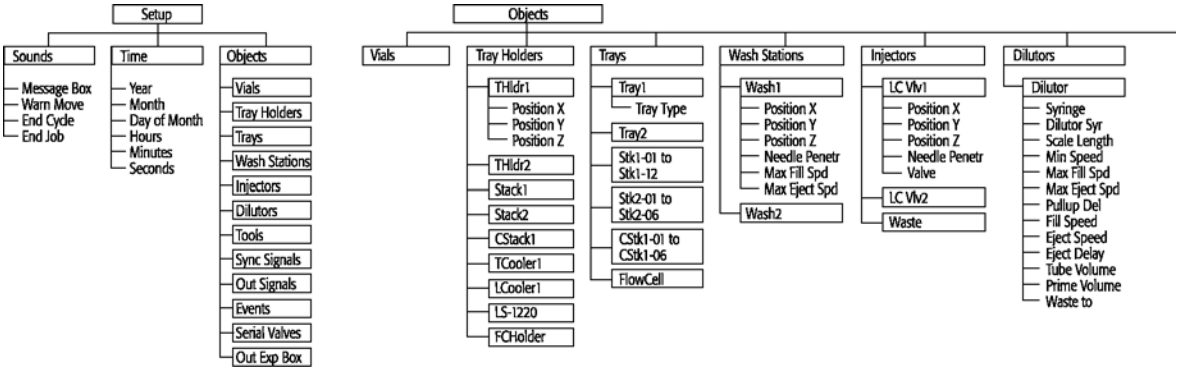


Figure 115 PAL HTS-xt Firmware Overview, Page 2

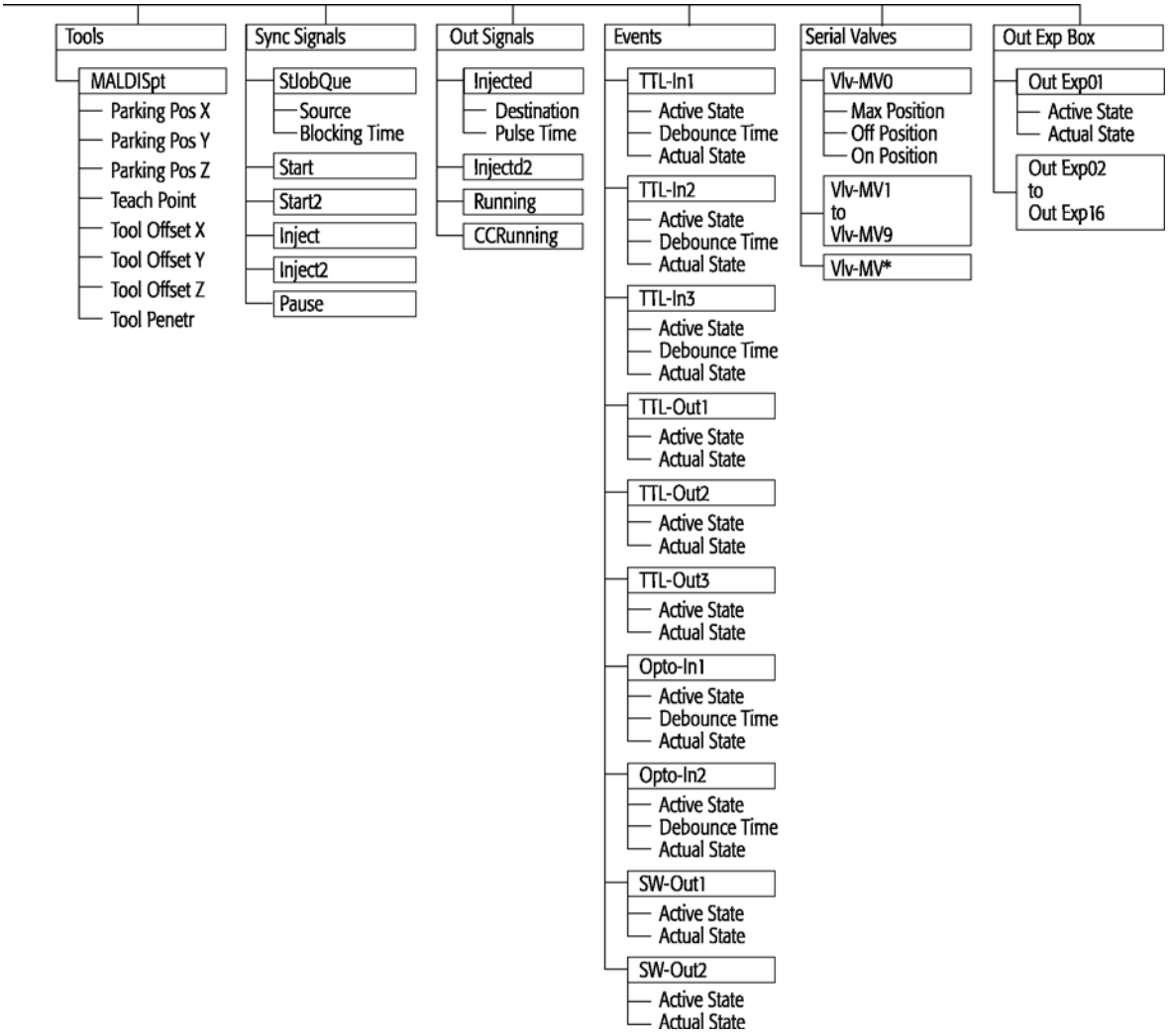


Figure 116 PAL HTS-xt Firmware Overview, page 3

Method Cycle	Recommended PAL Method Parameters
LC-Inj	
CYCLE	LC - Inj
SYRINGE	100 µl
Sample Volume	80 µl
Air Volume	0 nl
Pre Cln Slv1	1
Pre Cln Slv2	0
Pre Cln Spl	2
Fill Speed	5 µl/s
Fill Strokes	5
Pullup Del	5-10 s
Inject to	LC Vlv 1
Inject Speed	5 µl/s
Pre Inj Del	500 ms
Pst Inj Del	500 ms
Pst Cln Slv1	2
Pst Cln Slv2	0
Vlv Cln Slv1	2
Vlv Cln Slv2	0

Method Cycle	Recommended PAL Method Parameters	Remarks
LC-Cut		
CYCLE	LC - Cut	
SYRINGE	100 µl	
Sample Volume	80 µl	
Air Volume	0 nl	
Pre Cln Slv1	1	
Pre Cln Slv2	0	
Pre Cln Spl	2	
Fill Speed	10 µl/s	
Fill Strokes	3	
Pullup Del	3-10 s	
Inject to	LC Vlv 1	
Inject Speed	10 µl/s	
Pre Inj Del	500 ms	
Pst Inj Del	500 ms	
SplTransfDel	Sample Transfer Delay	Time needed to transfer sample from Loop onto 2nd valve (pre - or analytical column)
Pst Cln Slv1	2	
Pst Cln Slv2	0	
Vlv Cln Slv1	2	
Vlv Cln Slv2	0	
Cut Valve	LC Vlv 2	Specify name of 2nd valve, switching valve
Cut Delay		Time needed for clean-up. Switch to backflush sample, to analytical column or detector
MinCondTime		Minimum Conditioning Time: Time needed to condition pre - column on 2nd valve

Example for the following conditions:

- Loop Size 20 µL, overfill 3 - 5 times
  Partial Loop filling: Allowed sample volume range 20 - 60% of loop content for loops ≤ 100 µL
  Larger Loops: 20 - 80% of loop content

- Syringe:
  Syr X G100 - 225 - 3: Gauge 225 limits fill speed to max 20 µL/s (5-20 µL/s depends on viscosity of solvent)
  Syr X G100 - 22 - 3: Gauge 22 allows fill speed up to 200 µL/s (depends on viscosity of solvent)

- Eject speed for 100 µL Syringe: 50 to 150 µL/s (Utilities/Syringe)

- Pre - and Post Washing:
  Use Solvent 1 and 2 for samples with components of extremly different polarities.
  Samples containing proteins should not contact organic solvents.

- Wash Steps for biological samples:
  1st Wash Cycle: Aqueous solvent
  2nd Wash Cycle: Organic solvent

  1st Wash Cycle before next sample:
  Pre-wash with aqueous solvent (eliminate organic solvents in Syringe and Valve)

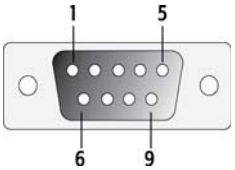
LC-Cut Cycle controls 2 valves.

LC Valve 1: Injection valve with loop.
LC Valve 2: Switching valve.
- Example 1: Pre - Column for sample clean-up, backflushing to analytical column. Cut Delay > 0.
- Example 2: 10 - port valve with 2 analytical columns. Loading column 1, condition column 2.
  Valve Toggle after cycle time. Loading column 2, condition column 1. Cut Delay = 0

Figure 117 PAL HTS-xt Firmware Overview, Page 4

External Connectors

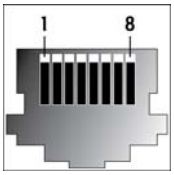
Connector SER1

Connector SER1	Pin	Signal Name
		
	1	NC
	2	RXD
	3	TXD
	4	DTR[1]
	5	GND
	6	NC
	7	RTS[2]
	8	NC
	9	NC

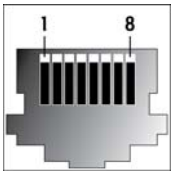
[1] Bridged with Pin7; Special grounding, do not alter.

[2] Bridged with Pin 4; Do not alter.

Connector TERMINAL or SER3

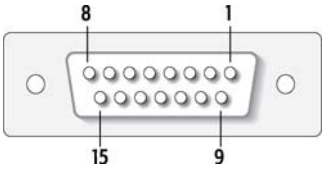
Connector TERMINAL or SER3	Pin	Signal Name
		
	1	GND
	2	RXD
	3	TXD
	4	NC
	5	NC
	6	+5 V
	7	NC
	8	GND

Connector SER2

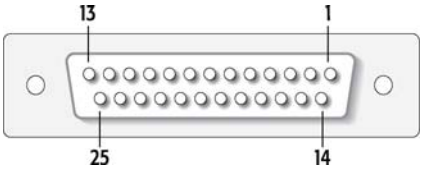
Connector SER2	Pin	Signal Name
		
	1	GND
	2	RXD
	3	TXD
	4	NC
	5	NC
	6	+5 V
	7	NC
	8	GND

Connector INTERFACE 1*

*APR Control-LC Injector HTC/HTS Board:"INTERFACE" and not "INTERFACE1"

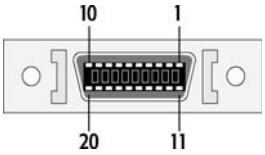
Connector INTERFACE 1	Pin	Signal Name
		
	1	Pwr-Out1 +
	2	Pwr-Out2 +
	3	SW-Out1 N0
	4	SW-Out1 COM
	5	SW-Out2 N0
	6	Opto-In1 +
	7	TTL-In1 +
	8	GND
	9	Pwr-Out1 -
	10	Pwr-Out2 -
	11	TTL-In2
	12	SW-Out2 COM
	13	TTL-In3
	14	Opto-In1 -
	15	+5 V

Connector INTERFACE 2

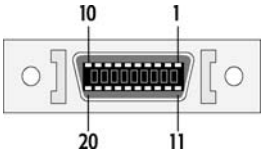
Connector INTERFACE 2	Pin	Signal Name
		
	1	TTL-In1
	2	TTL-In2
	3	TTL-In3
	4	TTL-Out1
	5	TTL-Out2
	6	TTL-Out3
	7	Opto-In1 +
	8	Opto-In2 +
	9	SW-Out1 NO
	10	SW-Out2 NO
	11	Pwr-Out1 +
	12	Pwr-Out2 +
	13	+5V
	14-19	GND
	20	Opto-In1 –
	21	Opto-In2 –
	22	SW-Out1 COM
	23	SW-Out2 COM
	24	Pwr-Out1 –
	25	Pwr-Out2 -

Connector AUX1*

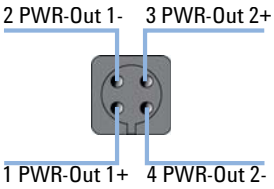
*APRControl-LC Injector HTC/HTS Board: ”AUX” and not “AUX1”

Connector AUX1	Pin	Signal Name
		
	1,2	Motor A1
	11, 12	Motor A2
	3, 4	Motor B1
	13, 14	Motor B2
	15, 18, 19	GND
	6	Temp +
	16	Temp -
	7	Sens
	17	+5 V
	8, 9	Heater
	10,20	+36 V

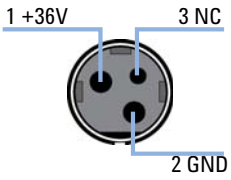
Connector AUX2

Connector AUX2	Pin	Signal Name
		
	1,2	Motor A1
	11, 12	Motor A2
	3, 4	Motor B1
	13, 14	Motor B2
	15, 18, 19	GND
	6	Temp +
	16	Temp -
	7	Sens
	17	+5 V
	8, 9	Heater
	10,20	+36 V

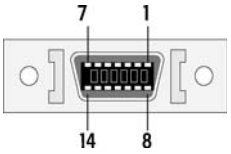
Connector Wash Station

Connector WASHSTATION	Pin	Signal Name
		
	1	Pwr-Out1+
	2	Pwr-Out1-
	3	Pwr-Out2+
	4	Pwr-Out2-

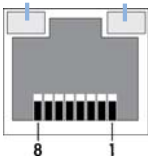
Connector Power

Connector POWER	Pin	Signal Name
		
	1	+36 V
	2	GND
	3	NC

Connector MODBUS

Connector MODBUS	Pin	Signal Name
		
	1	TXD
	2 — 7	+36 V
	8	RXD
	9 — 14	GND

Connector LAN

Connector LAN	Pin	Signal Name	DIR	Primary Function
LED left LED right 				
	1	TX+	Out	Transmit Data +
	2	TX-	Out	Transmit Data -
	3	RX+	In	Receive Data +
	4, 5	Not used		Terminated
	6	RX-	In	Receive Data -
	7, 8	Not used		Terminated

Connector Fuse

Fuse Type	FST 5x20
Rating	6.3 A
Rated	250 VAC

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U

V

W

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In This Book

The manual describes the following:

- Introduction
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- Operating
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- Maintenance
- Replacing Parts
- Limitations
- Appendices

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