myAntec®

ALEXYS AS 100

Autosampler

user manual

181.0010, Edition 3, 2009

Antec .... Proven Performance!

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CE

DECLARATION OF CONFORMITY

The manufacturer hereby declares that the product

ALEXYS AS 100 auto sampler	type 181

To which this declaration relates, is in conformity with the following directives:

EEC directives 89/392, 91/368 and 93/44 (machine safety) and EEC directives 73/23 and 93/68 (low voltage safety):

Safety requirements for laboratory equipment (Class I, Installation cat. II, Pollution degree 2)	EN61010-1

EEC directives 89/336 and 92/31 (EMC requirements):

EMC requirements for electrical equipment for measurement, control and laboratory use	EN 61326-1
Emission- Industrial, Scientific and Medical (ISM) equipment	EN 55011 (Class B)
Harmonic current emissions	EN 61000-3-2
Voltage fluctuations and flicker	EN 61000-3-3

Attention

Use manufacturer-supplied cable(s) only to connect all I/O's with other devices. Thoroughly connect the shielding to common. Manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices, which do not meet relevant safety standards.

February 21, 2007

Intended use

For research purposes only. While clinical applications may be shown, this instrument is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.

WEEE directive

All equipment of Antec Leyden which are subjected to the WEEE directive shipped after August 13, 2005 are compliant with the WEEE marking requirements. Such products are labelled with the “crossed out wheelie”, depicted on the left site.

The symbol on the product indicates that the product must not be disposed as unsorted municipality waste.

Collection & recycling information

Please ship the instrument back to the manufacturer (Antec Leyden, the Netherlands) at the end-of-life time of the product. The manufacturer will take care of the proper disposal and recycling of the instrument at its facilities.

Shipping address for the end-of-life products:

Antec Leyden
Industrieweg 12
2382NV Zoeterwoude
The Netherlands

In case of questions, or if further information is required about the collection & recycling procedure, please contact your local distributor.

ROHS directive

Our instruments are currently exempt from the RoHS directive because they fall under WEEE Annex IA categories 8 and 9, which includes medical devices and monitoring and control instruments. Nevertheless, we have taken steps to eliminate all restricted substances from our products.

Antec Leyden is an ISO 9001:2000 certified company.

About this manual

This manual has been written for laboratory technicians who use the AS 100 for execution of analytical runs. It is assumed that the user of this manual has basic knowledge of how to use menu-driven software and that she/he is familiar with standard laboratory and HPLC terminology.

Chapters 1 and 2 of this manual contain basic information that should be read by every user of the AS 100:

- Chapter 1 identifies main parts of the AS 100.
- Chapter 2 explains how to install parts either for maintenance purposes or for preparing the AS 100 for an analytical run.

For first-time users:

- Chapter 3 explains the main menus of the AS 100 and offers a basic explanation of how to operate the AS 100.
- Chapter 4 offers a number of examples that teach the user how to work with the AS 100.

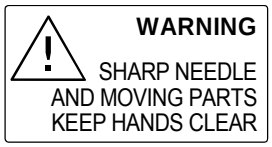
For experienced users:

- Users who understand how the AS 100 works will probably only use the reference part of this manual (Chapter 5) to look up the purpose of a particular function.
- Experienced users may find the overview of programming options (Appendix G) useful for quick reference of possibilities of the AS 100.

The appendices in this manual offer specialist information.
An index has been provided to allow the user to find required information quickly.

Symbols

The following symbols are used on the AS 100:



This sticker indicates that care should be taken to prevent personal injury or damage to parts of the AS 100.

This sticker (with yellow background colour) at the back of the AS 100 calls attention to the fact that you are expected to consult this manual for instructions on how to operate the AS 100.

The following pictograms are used in this manual:

calls attention to a procedure, which, if not correctly executed, could result in injury or loss of life. Do not proceed beyond a "DANGER" sign until the indicated conditions are fully understood and met.

calls attention to a procedure, which, if not correctly executed, could result in personal injury. Do not proceed beyond a "WARNING" sign until the indicated conditions are fully understood and met.

calls attention to a procedure, which, if not correctly executed, could result in damage to the equipment. Do not proceed beyond a "CAUTION" sign until the indicated conditions are fully understood and met.

calls attention to important information. Read this information before continuing.

Safety practices

The following safety practices are intended to ensure the safe operation of the equipment.

- Removal of panels may expose users to dangerous voltages. Disconnect the AS 100 from all power sources before removing protective panels.
- Always replace blown fuses with fuses of the size and rating indicated on the fuse panel and holder. Refer to Appendix B of this manual for more information on fuses.
- Replace or repair faulty insulation on power cords.
- Check that the actual power voltage is the same as the voltage for which the AS 100 is wired. Make sure power cords are connected to correct voltage sources.
- The AS 100 must only be used with appliances and power sources with proper protective grounding.

- Perform periodic leak checks on supply lines.
- Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable and/or toxic solvents through the municipal sewage system
- Using the AS 100 in other ways than indicated in the instructions given in this manual may cause unsafe conditions.

Table of contents

C H A P T E R 1

Introduction

The AS 100 autosampler utilises the proven concept of a rotating sample tray and a fixed sampling needle arm. This rugged concept guarantees uninterrupted and reproducible sampling.
The AS 100 offers considerable flexibility in vial choice (dimensions) and in programming injection sequences, complete with mixing, and washing steps. Several needles for optimising reproducibility and accuracy are available, for example our fused silica needle is unique. Sample tray cooling (down to 4 ^{o}C) further improves method reproducibility.
The sampling needle is inside a protective pre-puncturing needle which even penetrates Eppendorf tubes without any damage to the needle.
PASA (Pressure Assisted Sample Aspiration) facilitates sampling through narrow bore tubing without any problems. Injection volumes can be as small as 1 µL and is possible without any sample loss (µL pick-up).

Injection principle

The AS 100 offers three different methods of injection for an analytical run:

Flushed loop	The sample loop is completely (quantitatively) filled with sample resulting in extremely good reproducibility.
Partial loop fill	The sample loop is partially filled with sample; this means low sample loss and programmable injection volumes.
µL pick-up	After aspiration of sample, the sample is transported into the loop with transport liquid (mobile phase); this means no sample loss.

The AS 100 uses a syringe to aspirate the sample from a vial into the sample loop. To prevent contamination of the syringe the AS 100 is equipped with buffer tubing between the syringe and the injection valve. Wash solvent is used to remove the sample from the buffer tubing and sample needle, and to rinse the buffer tubing and sample needle. For more technical information on the injection principle used by the AS 100 refer to Appendix H.

For an overview of fluid connections of the AS 100 refer to the illustration inside the cover of the AS 100.

Front view of the AS 100

The front of the AS 100 contains the following elements:

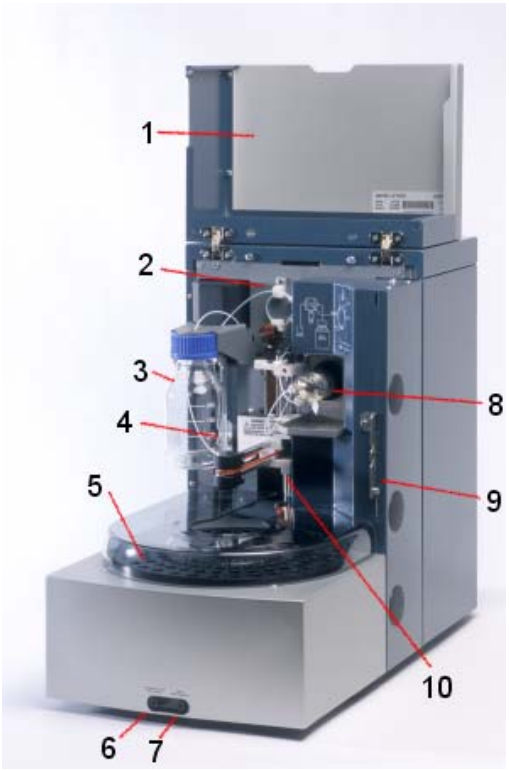


Fig. 1. Front view of AS 100.

1. Cover (in open position)
2. Buffer tubing
3. Wash solvent bottle
4. Syringe (behind bottle)
5. Tray with segments
6. Condensed water and leakage
7. Drain wash-position
8. Injection valve
9. Tubing holder
10. Needle unit at wash position

Back view of the AS 100

The back of the AS 100 contains the following elements:

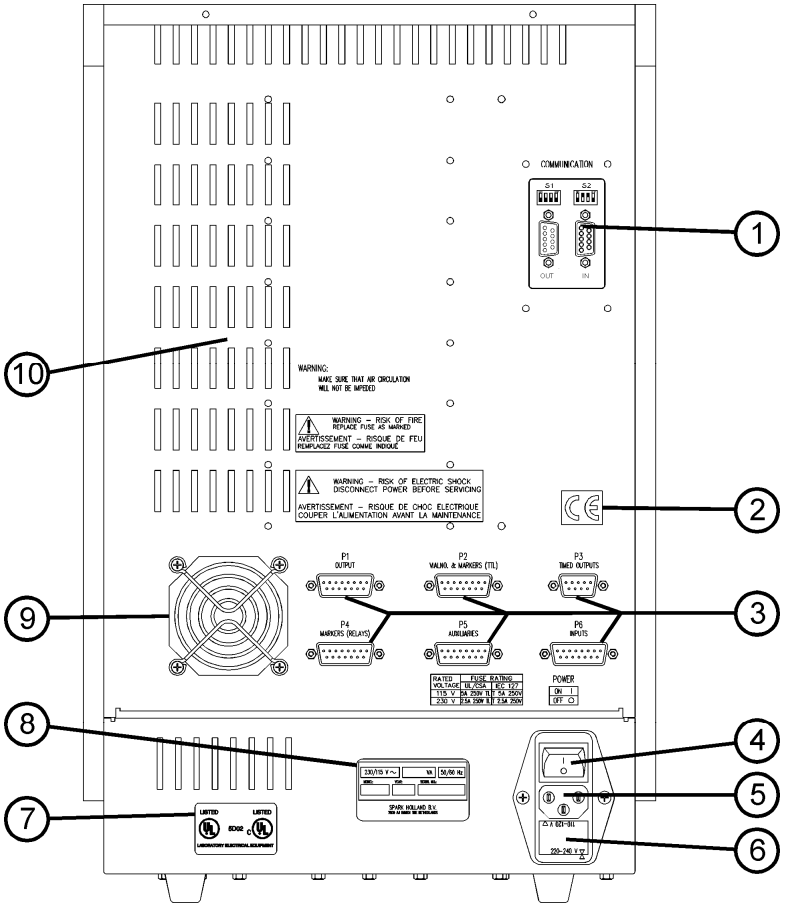


Fig. 2. Back of the AS 100

1. Communication interface connector.
2. CE-mark
3. I/O connectors
4. Mains switch
5. Mains input
6. Fuses and voltage selector
7. UL label
8. Type label
9. Fan (if tray cooling is installed)
10. Ventilation holes

Keypad and display of the AS 100

As a standard the AS 100 comes without keypad. However, for service purposes a keypad is available. The keypad of the AS 100 contains the following elements:

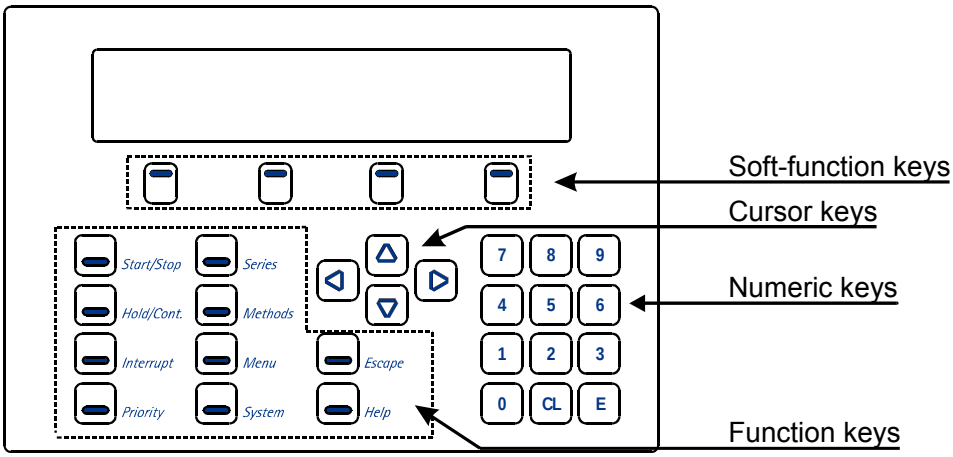


Fig. 3. For service only: keypad and display

Soft function keys: the label assigned to these keys depends on the menu that is active. The label of each key is shown in the bottom line of the display.

Cursor keys: can be used to move to a different field in the display, to move to a different field in a menu, or to make a displayed value higher or lower.

Numeric keys:

0 to **9**: to enter numerals in the various programming fields.

CL: to clear a value in a field or replace it by NONE or AUTO.

E: stands for Enter; to go through menu lines or to confirm a choice made in a menu or a value entered. The entered value is checked for validity and then saved.

Function keys:

Run control keys:

Start/Stop: to start or stop automatic processing, or to reset the system after an error has occurred.

Hold/Cont.: to hold or continue the analysis time. The analysis time is extended by the period that Hold is active.

Interrupt: not used.

Priority: to stop a run to process a priority sample before analysing the rest of the programmed sample series. Before the run is interrupted processing of the present sample will be finished. As soon as the priority sample has been analysed, the analytical run is resumed. A priority sample is a series of one vial with an injection method, a wash method and a time base method defined in a template (only possible if the correct settings are entered in the System Menu).

Programming keys:

Series: to enter the Series Menu in which series can be defined for an analytical run.

Methods: to enter the Methods Menu in which methods can be programmed for use in an analytical run.

Menu: this key can only be used if [MENU] or [MN] is shown in the top right hand corner of the display. If pressed more possibilities of the menu are displayed.

System: to enter the System Menu in which system settings can be entered.

General keys:

Escape: allows the user to leave the programming mode or go to a previous level in the menu. Entered values are checked for validity and then saved.

Help: to display help information; available only for a limited number of functions!

C H A P T E R 2

Preparations for use

This chapter describes procedures for replacement or installation of parts, either for maintenance or for preparation of the AS 100 for an analytical run.
A number of items required for use of the AS 100 are factory-installed (see Appendix A). However, after the installation procedure described in Appendix A has been executed, check that the following have been correctly installed before you start to use the AS 100:

- HPLC pump & column connections
- waste tubing
- wash solvent bottle
- syringe
- needle assembly
- syringe, sample loop and buffer tubing
- tray segments and tray types
- reagent vials, transport vials
- AS 100 driver is loaded and functional in ALEXYS data system

AS 100 Control

After installation of the software with device drivers full functionality of the AS 100 can be controlled from ALEXYS data system (Fig. 4).

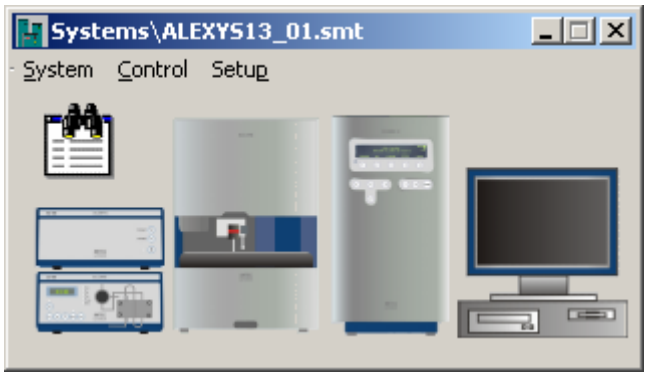


Fig. 4. ALEXYS data system with AS 100 driver installed.

Double clicking on the autosampler icon shows the AS 100 control window. In this manual references are made from this point. For example,

switching the valve to load will be described as: switch valve to load by clicking ‘Load’ button in ‘Manual/Main/Injector valve’ menu.

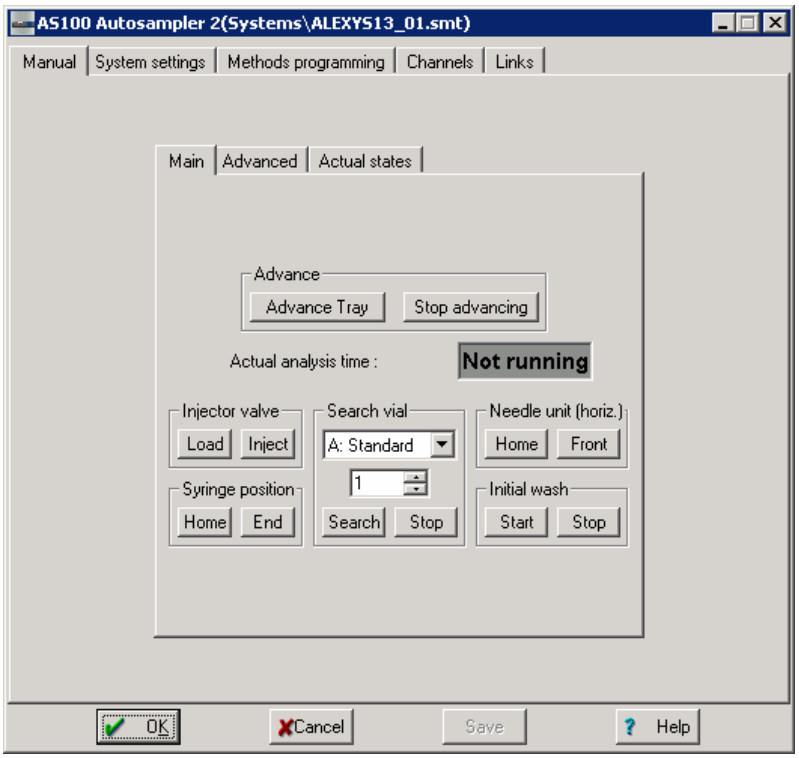


Fig. 5. AS 100 driver in ALEXYS data system, ‘Main’ page for manual control of autosampler.

HPLC connections

Make the following connections:

- HPLC pump to port 1 of the injection valve
- HPLC column to port 6 of the injection valve

The instrument has been flushed with isopropanol before dispatch from the factory. Make sure that the mobile phase of your HPLC system is miscible with isopropanol, or start up with an intermediate solvent as mobile phase (disconnect the HPLC column).

The contents of the sample loop are injected in back flush onto the column, therefore: do not exchange column and pump connections at the injection valve.

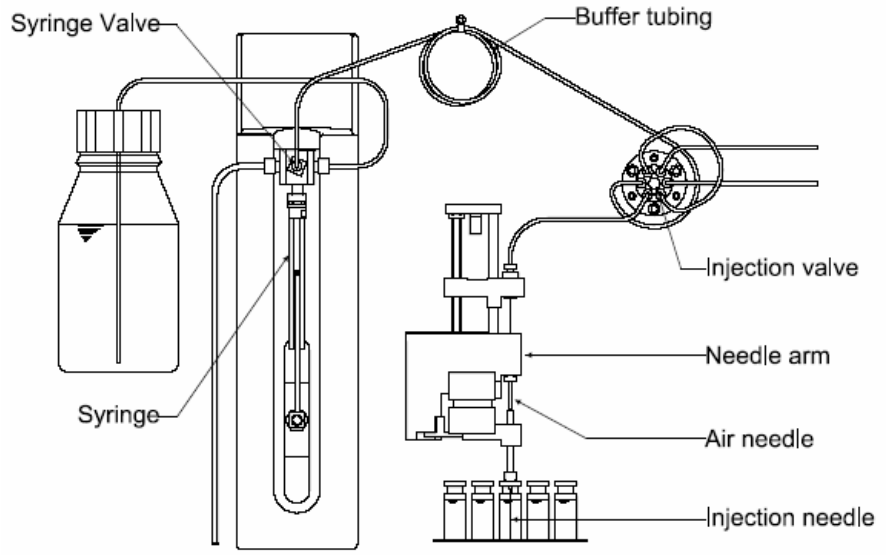


Fig. 6. Wash solvent bottle, syringe, needle and injection valve.

Waste tubing

Make the following connections (see Fig. 1):

- Syringe waste: put the end of the syringe waste tube in a bottle placed next to the AS 100.
- Drain wash-position: connect the hose to the drain wash connector of the AS 100. Place the other end of the hose in a bottle placed on the floor. Through this drain all liquid dispensed to waste at the back of the tray is removed.
- Condensed water and leakage: connect the hose to the drain port of the AS 100. Place the other end of the hose in a waste container on the floor. Through this hose all leakage solvents and condensed water (if cooling is installed) are drained.

Make sure that the flow path of the hoses is not obstructed in any way.

Wash solvent

The AS 100 has a 250 mL wash solvent bottle. Execute the following steps to install the 250 mL wash solvent bottle:

1. Fill the wash solvent bottle with the appropriate wash solvent. Use of methanol (organic solvent, no buffers) or a mixture of water and isopropanol (80/20) is recommended. Before using the wash solvent, degas it with Helium or an ultrasonic bath.
2. Screw the bottle to the cap in the holder.

3. Place the holder in the AS 100 as indicated in Fig. 6.
4. Put the wash solvent tube in the wash solvent.
5. Lower the cover of the AS 100.
6. Click ‘End’ button in ‘Manual/Main/Syringe position’ menu to fill the syringe (Fig. 5).
7. Place the syringe back in home position by clicking ‘Home’ button.
8. Repeat steps 6 and 7 until the wash solvent tube and the syringe are completely filled.
9. Click ‘Start’ button in ‘Manual/Main/Initial wash’ menu to perform a standard wash routine.
10. If any air remains in the syringe, click ‘End’ button again to fill the syringe with wash solvent; click ‘Home’ again to move contents to waste. Repeat if there is still air in the syringe and gently tap the syringe as wash solvent is dispensed to waste.

If you use an application that requires more than 250 mL of wash solvent for a complete run, install a longer tube (with flanged end for valve fitting) and place a larger bottle next to the AS 100. To fill the wash solvent tube, you may have to repeat the above-mentioned filling procedure (steps 6 and 7) a few times.

Syringe

The AS 100 is supplied with a 250 µL syringe. It is also possible to use the AS 100 with a 100µL, 500 µL, 1000 µL or 10 mL syringe. Execute the following steps to install a syringe:

1. Click ‘End’ button in ‘Manual/Main/Syringe position’ menu (Fig. 4) to move the syringe to end position.
2. Lift the cover.
3. Unscrew the top of the syringe (turn clockwise).
4. Pull the bottom of the syringe towards you; you can now remove the syringe (refer to Fig. 7).
5. Fill the new syringe with wash solvent and make sure that all air bubbles are removed from the syringe.
6. Connect the bottom of the filled syringe to the AS 100.
7. Screw the top of the filled syringe to the AS 100 (counter clockwise).
8. Lower the cover.
9. Click ‘Home’ button to remove air from the syringe. The syringe moves to home position and its contents are dispensed to waste.
10. If any air remains in the syringe, Click ‘End’ button again to fill the syringe with wash solvent; Click ‘Home’ button again to move contents to waste. Repeat if there is still air in the syringe and gently tap the syringe as wash solvent is dispensed to waste.

11. Click ‘Start’ button in ‘Manual/Main/Initial wash’ menu to execute a standard wash routine. All tubing connected to the syringe valve is filled and rinsed.

Select the appropriate syringe in the ‘System settings/General/Syringe volume’ menu and install the correct buffer tubing.

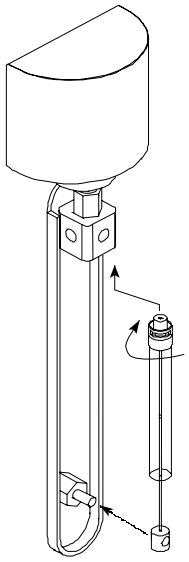


Fig. 7. Replacing the syringe

Needle assembly

The needle used for sampling consists of two parts:

- pre-puncturing needle: a hollow needle used for puncturing of the septum, capmat or sealer; also used to put headspace pressure on the sample (approximately 0.5 bar).
- sample needle: placed inside the hollow pre-puncturing needle; used for the actual transport of sample. Different types of needles can be used here (refer to options mentioned in Appendix D). If a needle with deviating diameter is used, a different air outlet nut (see Fig. 8 [6]) must be used that matches the injection needle.

Most commercially available sealers or capmats cannot be used in combination with headspace pressure. You are advised to switch off headspace pressure in those cases (‘System settings/General/Headspace pressure’ = no).

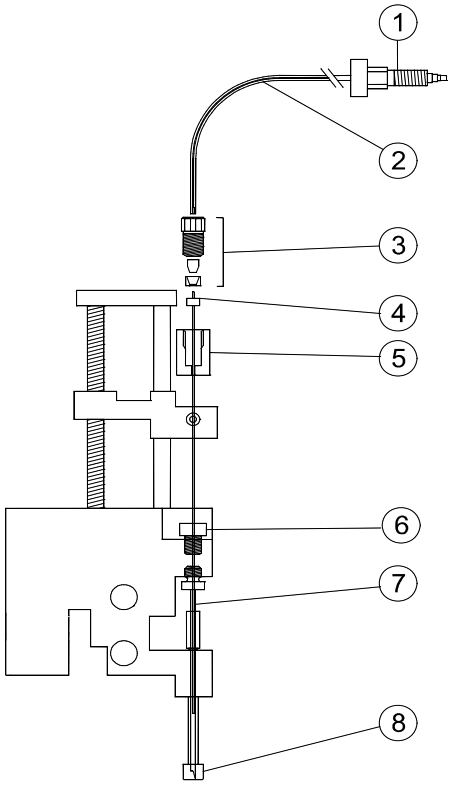


Fig. 8. Needle assembly

The following elements can be identified in the needle assembly (Fig. 8):

1. Nut and ferrule
2. Needle tubing
3. Needle connection nut
4. Standard sample needle
5. Needle holder
6. Air outlet nut
7. Pre-puncturing needle
8. Sensor (detects tray height, missing tray and missing vials).

Numbers 1, 2, 3, 4, and 6 constitute the sample needle.

Execute the following steps to replace a needle:

1. Loosen the needle connection nut (3).
2. Loosen the ferrule and nut (1) .
3. Carefully pull out sample needle and tubing.
4. Insert a new sample needle and tube through the needle holder (5) and tighten the nut (4).
5. Connect the other end of the tube to port 4 of the injection valve using the correct type of nut and ferrule. Do not tighten too much to prevent block of tubing.
6. Lower the cover of the AS 100.
7. Check sample needle height (default height: 2 mm). If necessary, adjust the value in the ‘System settings/General/Needle height’.
8. Click ‘Start’ button in ‘Manual/Main/Initial wash’ menu to clean the new sample needle.

Optional Needles

Needles of the following types can be installed in the AS 100:

• LSV needle	sample needle with large inner diameter for viscous samples, or in case large volumes are loaded in the loop
• PEEK needle	biocompatible needle
• Fused silica needle	needle with small inner diameter for small µL sampling from small volumes
• Extended needle	needle with extended tubing for switching valve placed in the side panel of the AS 100
• Silica-coated needle	needle for use with liquids that would be damaging to a stainless steel needle

Refer to Appendix D for an overview of options available for the AS 100.

Execute the following steps to install any of these needles:

1. Remove needle as described on page 13.
2. Remove the standard air outlet nut (Fig. 8 [6]) and replace it by the nut supplied with the optional sample needle.
3. Install optional sample needle as described in previous section.
4. Adjust settings in 'System settings/General/Needle tubing' to the volume of the new needle tubing.

Combination of syringe, sample loop and buffer tubing

The 250 µL syringe is the standard syringe; combined with the standard 500 µL buffer tubing and the standard 100 µL sample loop, the following injection volume range is available for the various injection modes:

Flushed loop	100 µL
Partial loop fill	1 - 50 µL
µL pick-up	1 - 27 µL

The maximum injection volumes (V_{max}) are calculated with the following formulas:

Flushed loop: V_{max} = loop volume
Partial loop fill: V_{max} = 50% of loop volume
µL pick-up: V_{max} = (loop volume - 3 x needle tubing volume) / 2

Five sizes of syringes can be used in the AS 100: 100, 250, 500, 1000 µL and 10 mL. The 10 mL syringe can only be used in the User Program (must be enabled in 'Methods programming/Template' menu) and not for the standard injection modes.

Flushed loop: maximum reproducibility
Partial loop: maximum accuracy
µL Pick-up: zero sample loss, also maximum accuracy

Flushed loop gives maximum reproducibility (RSD < 0.3%), but not maximum accuracy, since loop volume is specified with an accuracy of ± 10%. Minimum sample loss = 230 µL (2 x loop overfill + flush volume for needle) for the standard 100 µL loop.
Partial loop fill gives maximum accuracy (depends on syringe accuracy) and reproducibility better than 0.5% RSD for injection volumes > 5 µL Minimum sample loss (Flush volume) = 30 µL

30 µL is the recommended minimum flush volume (combined with an air segment); smaller flush volumes can be programmed, but will result in decreased performance.
µL Pick-up means zero sample loss, maximum accuracy (same as partial loop fill), but slightly diminished reproducibility: RSD better than 1% for injection volumes > 5 µL.
5 µL of air is injected together with the sample, if in 'System settings/General/Air segment' is selected in the System Menu.

For some cases other combinations of syringe, loop and/or buffer are advised:

Injection volumes smaller than 5 µL:

- Partial loop fill: use a 100 µL syringe for maximum reproducibility and accuracy. Use a 20 µL sample loop to avoid loss of accuracy due to expansion of the loop content when switching from inject to load position prior to sample loading. Specially when working with high pressure (200 bar), this loss may be 0.1 - 0.5 µL for a 100 µL loop. Note that the minimum sample loss in partial loop fill mode is 30 µL (recommended minimum flush volume) for the first injection and an additional 15 (always half the programmed flush volume) for additional injections from the same vial. If a wash between injections has been programmed, sample loss is 30 µL for every injection. For zero sample loss injections, use the µL-pick injection mode.

- µL Pick-up: use a 100 µL syringe for optimum accuracy and reproducibility. Do not use a smaller sample loop! The sample plug is transported into the loop with a plug of transport liquid, which equals 2.5 times the programmed needle tubing volume. You are advised to select 'System settings/General/Air segment = no' with µL pick-up.

Injection volumes up to twice the standard:
With the standard 250 µL syringe, standard needle with tubing (15 µL) and standard 500 µL buffer, but with a 200 µL sample loop, the maximum injection volumes are:

Flushed loop	200 µL (sample loss remains 230 µL since loops > 100 µL need only one loop volume overfill; 30 µL pre-flush)
Partial loop fill	100 µL
µL Pick-up	77 µL

Volumes smaller than 5 µL may be injected, but reproducibility and accuracy may not be < 0.5% for partial loop fill or < 1% for µL pick-up.
In short: loop < 100 µL: loop has to be filled three times
loop ≥ 100 µL - 499 µL: loop has to be filled twice
loop ≥ 500 µL: loop has to be filled 1.5 times

For volumes larger than 200 µL:
Use the 2000 µL buffer tubing, use the appropriate sample loop size and the appropriate syringe: Syringe volume > 2 x injection volume. Injection volumes larger than 500 µL are possible, but the sample may contaminate the syringe. Program sufficient wash after use!

Overview of appropriate buffer tubing for each type of syringe:

Syringe	Buffer tubing
100 µL	500 µL
250 µL	500 µL
500 µL	2000 µL
1000 µL	2000 µL
10 mL	15 mL

The 10 mL syringe and the 15 mL buffer tubing can only be used in combination with the User Program and not with the standard injection methods.

Tray segments and Vial types

A wide range of vials and septa can be used for the AS 100, from micro vials (0.5 mL) to super LSV vials (10 mL). Using inserts can reduce the volumes of standard vials.
The AS 100 can be equipped with four types of tray segments to accommodate use of the various types of vials (see Fig. 9).

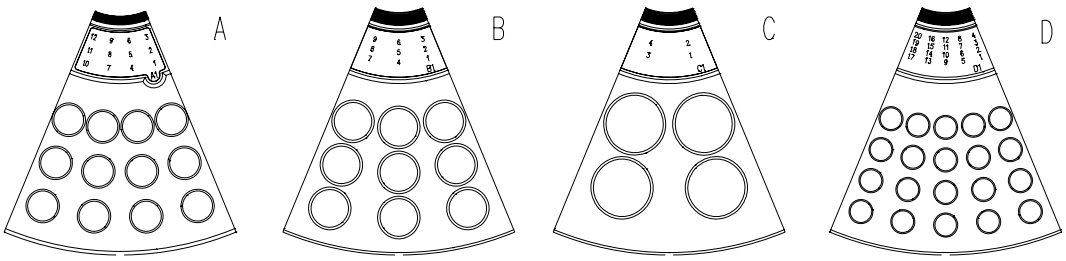


Fig. 9. Types of tray segments. Type A: Standard tray segment. Type B: LSV tray segment. Type C: Super LSV tray segment. Type D: Micro vial tray segment.

The AS 100 allows you to use more than one type of segment in a tray. Enter the configuration of the tray in the 'System settings/Tray configuration'.

The following vial types can be used:

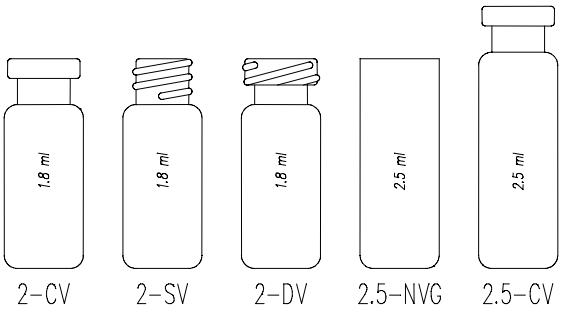


Fig. 10. Chromacol vials for standard tray (outer vial diameter :12mm)

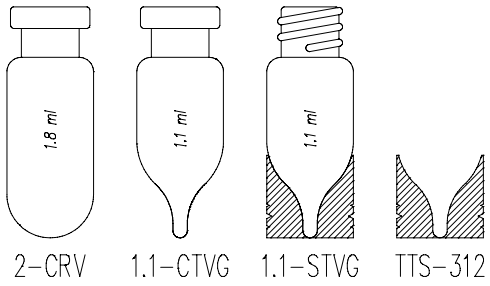


Fig. 11. Chromacol conical vials with support

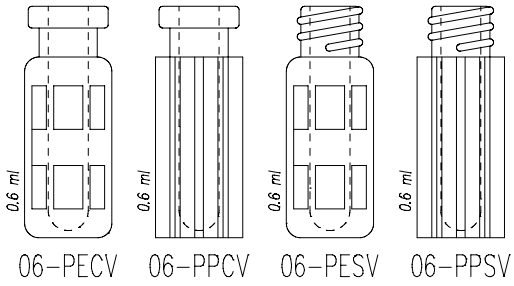


Fig. 12. Chromacol plastic vials

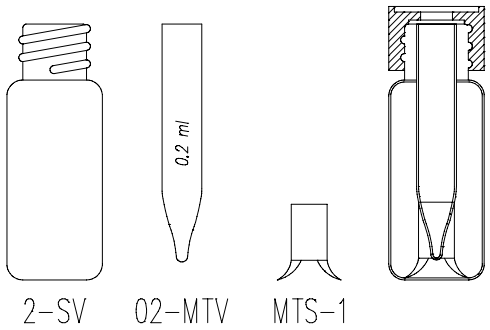


Fig. 13. Chromacol inserts (02.MTV, 02-MTVWG, 03-MTV) can be used in combination with the appropriate vial and support sleeve or spring.

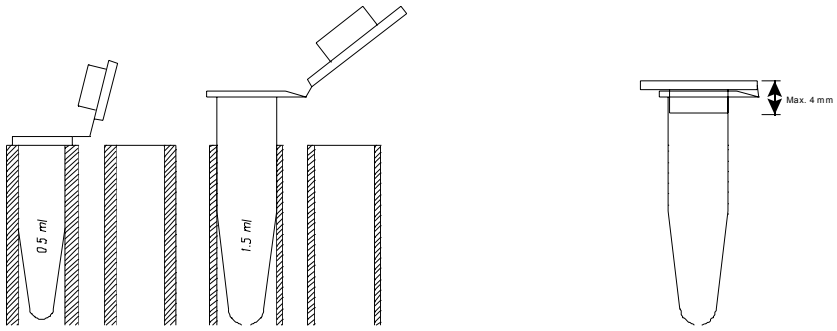


Fig. 14. Eppendorf micro centrifuge tubes with support sleeve.

Eppendorf micro centrifuge tubes can be used with a support sleeve. However, the pre-puncturing needle may not be able to pierce the caps of some brands of tubes because of the depth of the caps. This may result in damage to the sample needle.

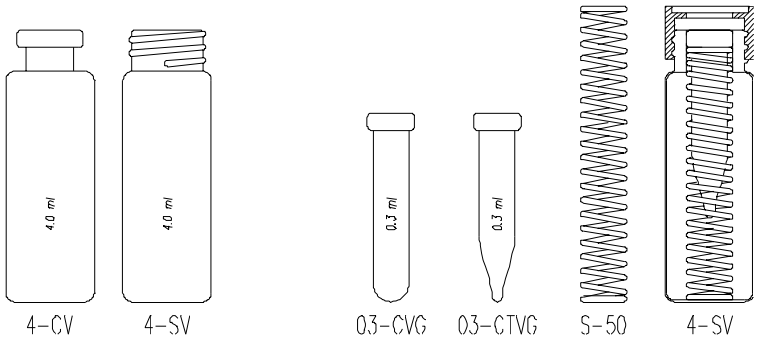


Fig. 15. Vials and inserts, Chromacol vials for LSV tray (outer vial diameter: 15 mm)

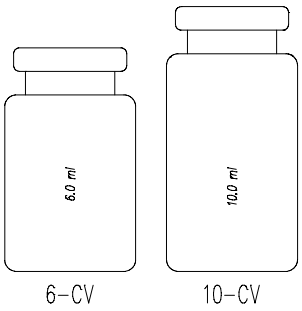


Fig. 16. Vials for super-LSV tray, Chromacol vials for super-LSV tray (outer vial diameter: 22 mm)

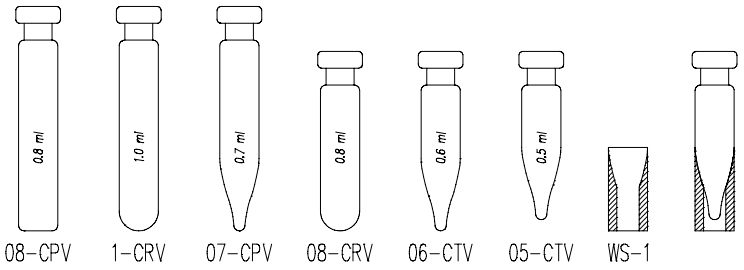


Fig. 17. Vials and support, Chromacol vials for micro tray (outer vial diameter: 7mm)

Vial handling

It is best to fill vials with a narrow-end pipette to allow air to escape when filling the vial. Do not fill vials to the edge to avoid that sample liquid will be forced into the air needle (risking cross-contamination of samples and fouling of the needles).

If you want to use headspace pressure for a sample handling routine, it is important to make sure that the seal is airtight. An airtight seal is also important to prevent air bubbles from forming and to prevent evaporation of volatile samples.
Check the seal after crimping; if the cap can be turned easily, the seal is not airtight and you will have to adjust the hand crimper.

Loading the sample tray

The tray segments can be placed in any position in the AS 100 tray. The tray segment types used must be defined in ‘System settings/Tray configuration’. Tray segments can only be placed in an open position in the front half of the tray. Click ‘Advance tray’ followed by ‘Stop advancing’ in ‘Manual/Main’ menu to rotate the tray if the AS 100 is not executing a run. You can then place a tray segment in the front half of the tray.

Do not place or remove a tray segment at the rear half of the AS 100 tray, as this may damage the tray sensors.

C H A P T E R 3

Getting started

This chapter describes how to get started with the AS 100 using ALEXYS data system. For keyboard support see appendix K and L. The following will be explained:

- menus of the AS 100
- convenient working order
- methods that can be programmed and linked to series
- executing a series.

Menus of the AS 100

Important menus in the software driver of the AS 100 are:

- **Manual**: for status and manual control of AS 100 such as valve and syringe position.
- **System settings**: for entering AS 100 parameters such as tray configuration, loop, needle tubing and syringe volume.
- **Methods programming**: for programming a method to be used in an analytical run.
- **Sample queue**: allows you to define a series of runs and to assign a 'system' to it which contains all relevant AS 100 settings and method.

Do not program conflicting injection volumes. If injection method is 'flushed loop' a full loop volume will be injected. Even if another volume is entered in sample queue editor!

Convenient working order

After you have determined what type of analytical run you want to perform, the most convenient working order for the AS 100 is:

1. Enter 'System settings/General' and 'Tray configuration' menu. Note that the settings for the AS 100 probably already have been correctly entered and otherwise enter correct settings.
2. Enter 'Methods programming/Methods' and program a method for the analyses you wish to perform.
3. 'Set' and 'Save' the system settings. In System window (Fig. 4) open 'System/Sample queue'.
4. click 'Edit' button in the queue window and define a series and link a 'system' with programmed method to a range of vials. Note that also

here the sample volume is entered, **be careful not to program conflicting values**.

5. 'Save' and exit the queue editor and click 'Start' button to execute the series.

Please note that it is allowed to use a different working order. Refer to chapter 5 for more information on specific items in menus and the way they influence the other menus.

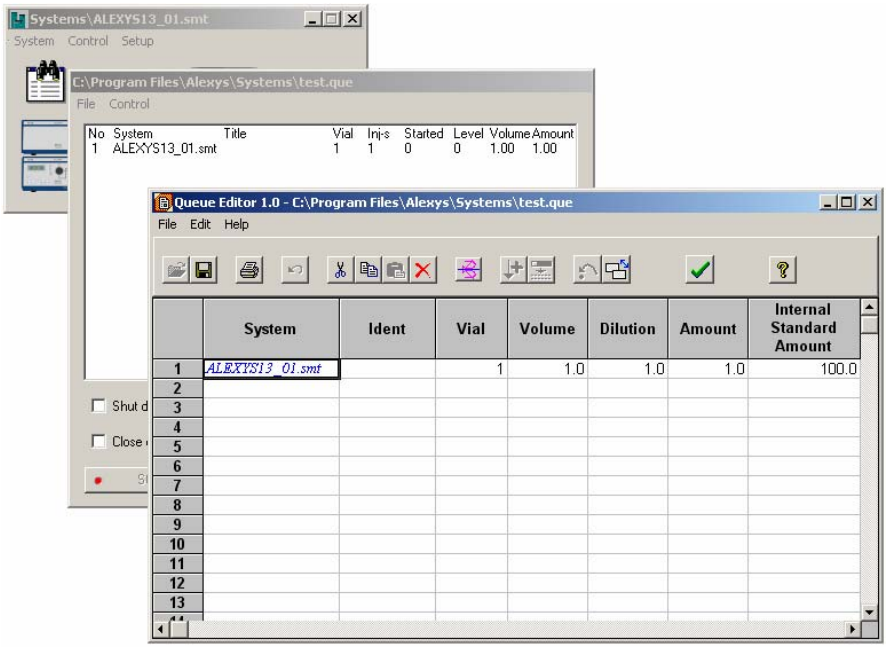


Fig. 18. System window, sample queue, and queue editor.

Types of Methods and links to Series

The AS 100 offers the following types of methods for different parts of the sample handling routine:

- **injection** method: contains information on the injection routine, flush volume and analysis time.
- **wash** method: describes a wash volume and when a wash must be executed.
- **mix** method: a pre-injection method in which additional sample handling can be performed (e.g. pre-column derivatisation).
- **time base** method: a post-injection method with which outputs to other devices (e.g. integrator or pump) and switching of the ISS valve are controlled.
- **user program**: offers the possibility to program sequences of all actions that can be executed by the AS 100 in separate steps.

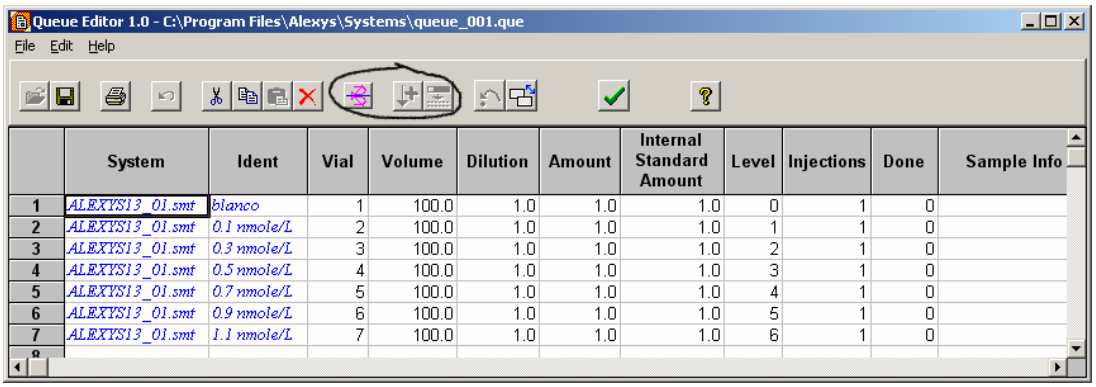
In ‘Methods programming/Templates’ the method(s) of choice must be selected. Finally a programmed method is saved with the system configuration. The ALEXYS data system offers the possibility to execute a combination of defined methods (systems) in a **sample queue**.

Executing a Queue

A queue is programmed in the queue editor:

1. Start programming the queue by clicking ‘System/Sample Queue’ (Fig. 18, top left corner) and clicking the ‘Edit’ button in the queue window.
2. Fill the queue in the editor. Note that in ‘System’ column different systems (with different methods) can be assigned.
3. Save and close the queue and click ’Start’ button to start the actual analytical run. The AS 100 starts to execute the series you have defined.

Refer to Chapter 4 for a number of examples illustrating this working order.



	System	Ident	Vial	Volume	Dilution	Amount	Internal Standard Amount	Level	Injections	Done	Sample Info
1	ALEXYS13_01.smt	blanco	1	100.0	1.0	1.0	1.0	0	1	0	
2	ALEXYS13_01.smt	0.1 nmole/L	2	100.0	1.0	1.0	1.0	1	1	0	
3	ALEXYS13_01.smt	0.3 nmole/L	3	100.0	1.0	1.0	1.0	2	1	0	
4	ALEXYS13_01.smt	0.5 nmole/L	4	100.0	1.0	1.0	1.0	3	1	0	
5	ALEXYS13_01.smt	0.7 nmole/L	5	100.0	1.0	1.0	1.0	4	1	0	
6	ALEXYS13_01.smt	0.9 nmole/L	6	100.0	1.0	1.0	1.0	5	1	0	
7	ALEXYS13_01.smt	1.1 nmole/L	7	100.0	1.0	1.0	1.0	6	1	0	

Fig. 19. Queue editor buttons ‘duplicate’, ‘increment’ and ‘propagate’ (circle) are particularly useful in filling the queue quickly.

If a series or method is changed during run, the new values become active the next time the AS 100 starts a series. The series currently running are not affected by the changes.

C H A P T E R 4

Using the AS 100

This chapter describes a number of examples of actions that can be performed with the AS 100 using ALEXYS data system. For keyboard support see appendix K and L. Please note that this chapter does not describe all types of actions that can be performed. Try to do these examples to learn to work with the AS 100.
These examples can be executed after the AS 100 has been installed in accordance with Appendix A and after all items described in Chapter 2 have been correctly set up.

Example 1: 10 µL partial loop fill injection

After the AS 100 has been switched on, an initialisation procedure is executed. In this example it is assumed that a loop of 100 µL, needle tubing of 15 µL, a syringe of 250 µL and a type A tray has been installed. Click on the autosampler icon in System window and enter settings:

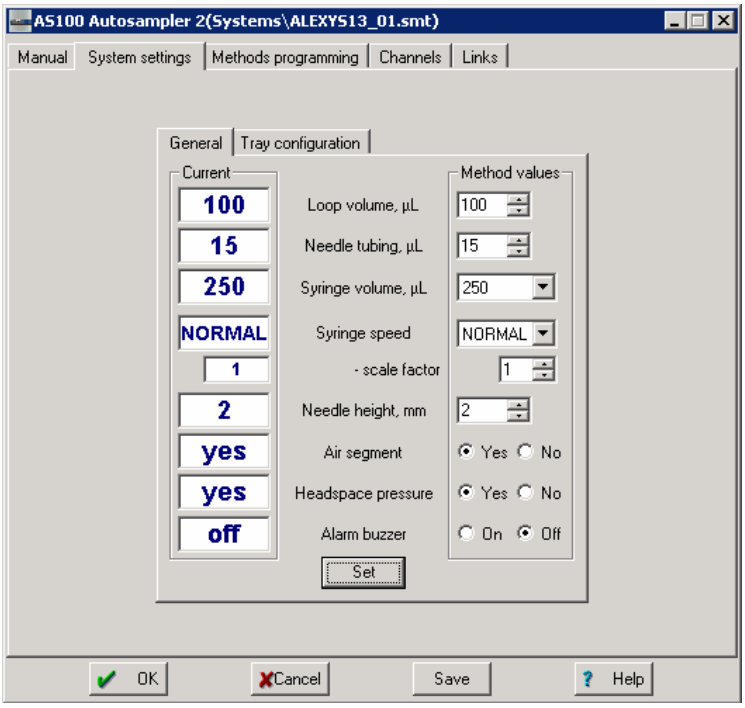


Fig. 20. System settings example 1.

Click ‘Set’ and ‘Save’ to save this configuration in the System. For this example all other settings used will be default.
Program the injection method and set template:

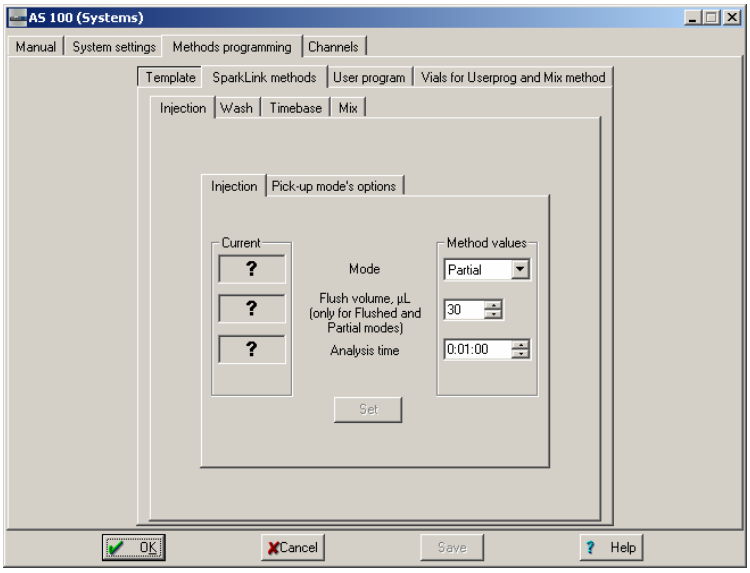


Fig. 21. Injection method example 1.

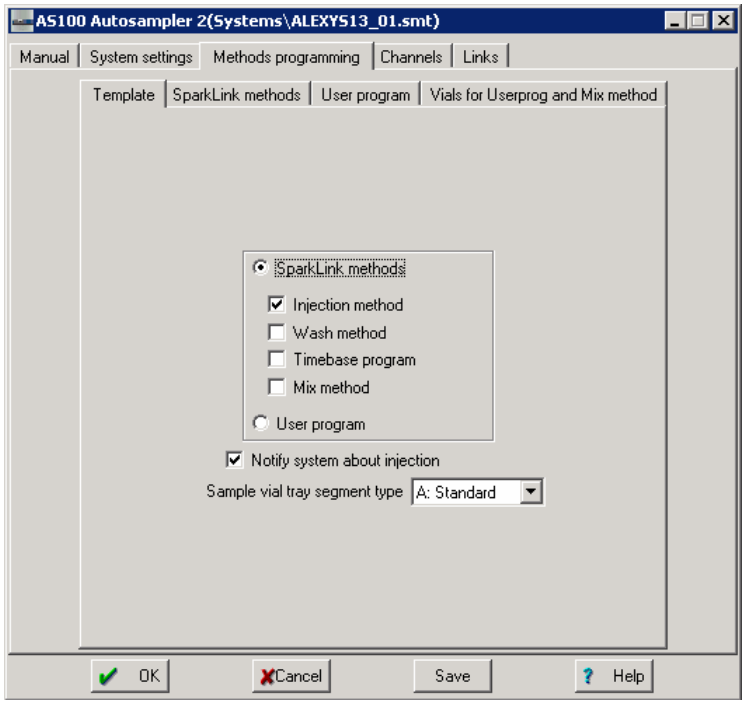


Fig. 22. Template settings example 1.

Make sure that sample tray position 1 contains the sample vial. Injecting the sample is done by selecting ‘Control/Start determination’ in the system window (Fig. 4). The system initialises and a ‘Edit sample description’ appears:

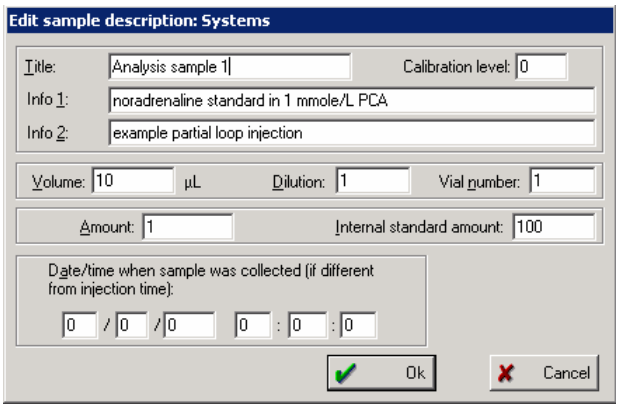
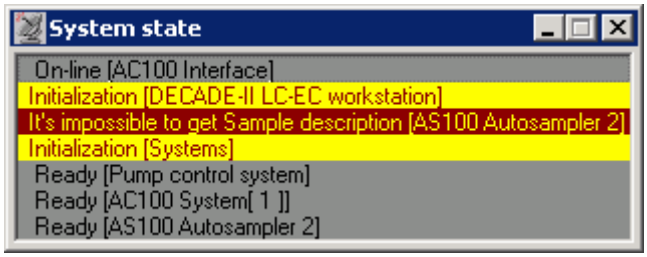


Fig. 23. Edit sample description, example 1.

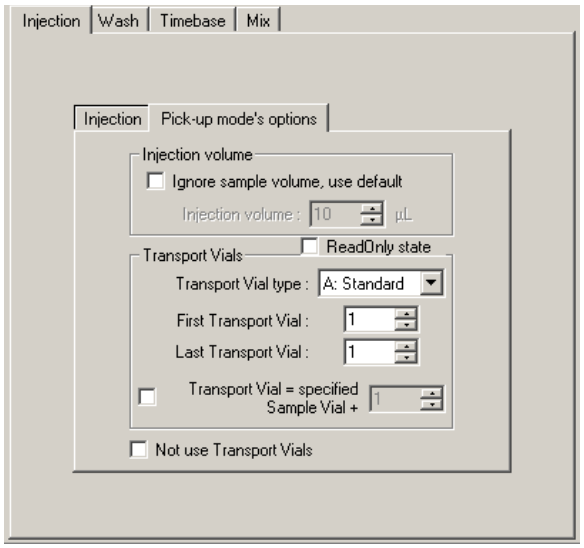
Note that the sample volume and vial number must be entered correctly in this window!

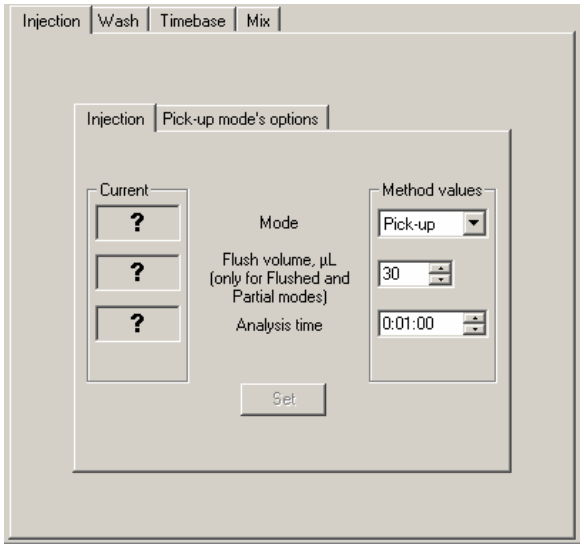
The AS 100 will now locate vial A 01 and perform a 10 µL partial loop fill injection. The System state window will indicate the status of ALEXYS 100 LC-EC system.



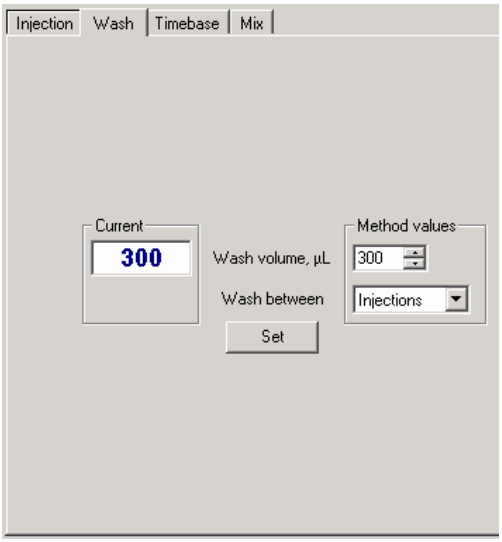
Example 2: 3 x 10 µL, µL pick-up, including wash

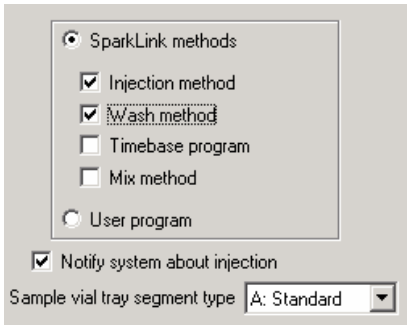
In this example a different injection method is used than in the previous one. In ‘System settings/General’ menu (Fig. 20) the air segment is set to ‘no’. In ‘Methods programming/Methods’ the transport vial for µL pick-up is set to position 1. Note that flush volume is not used for Pick-up methods.

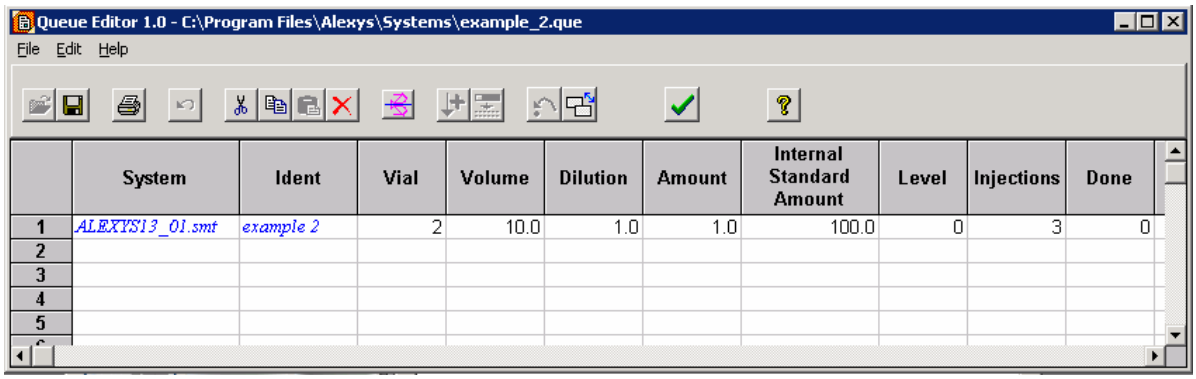




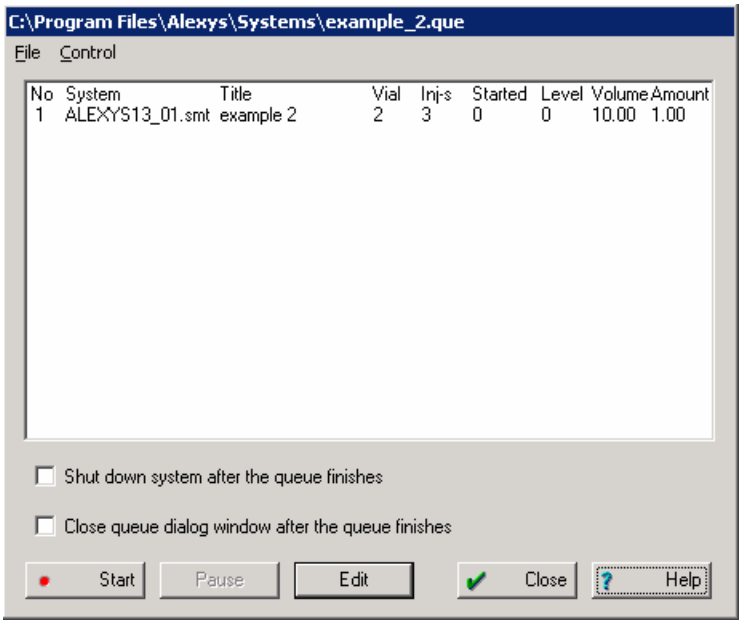
In ‘Wash’ a 300 µL washing step between injections is programmed, in Template (Fig. 22) also the Wash method is checked. In the queue editor 3 injections of 10 µL from sample vial number 2 are programmed. **Note that injection volume must be entered again correctly**.





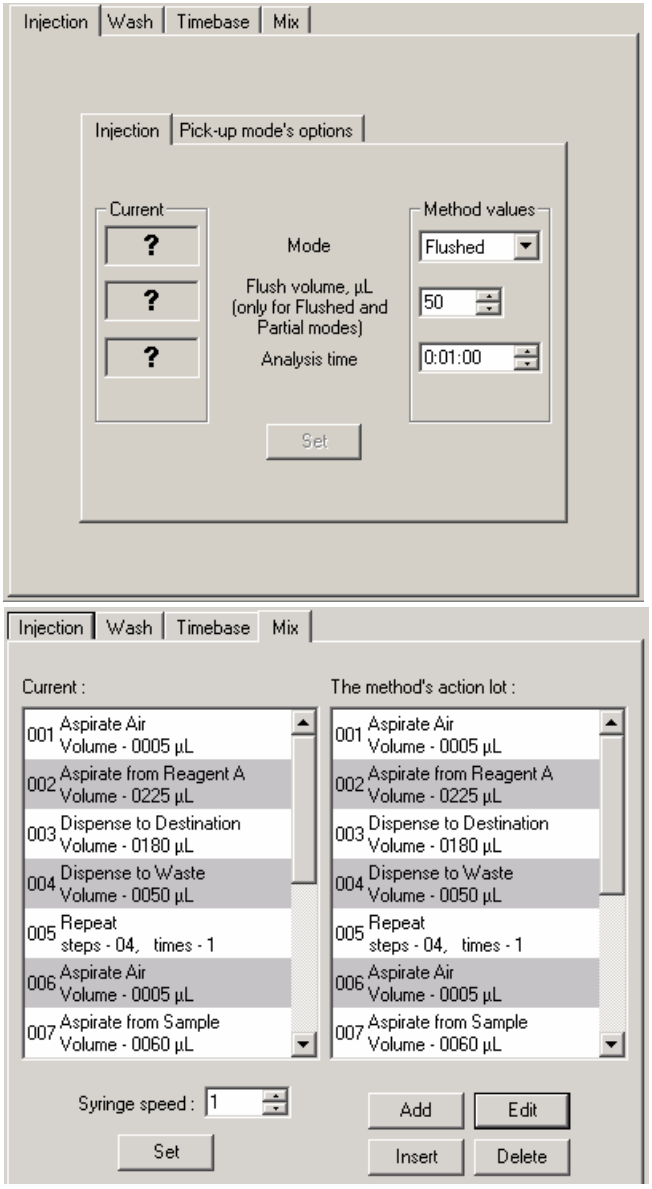


To run the queue, put a vial with transport solvent (mobile phase) in tray position A 1 and a vial filled with sample in tray position A 2. Make sure the transport vial is correctly filled before starting a new series. Click ‘Start’ button in queue window to start the sequence.


C:\Program Files\Alexys\Systems\example_2.que
File
Control
No System Title Vial Inj-s Started Level Volume Amount
1 ALEXYS13_01.smt example 2 2 3 0 0 10.00 1.00
Shut down system after the queue finishes
Close queue dialog window after the queue finishes
Start
Pause
Edit
Close
Help

Example 3: 10x dilution, 10 µL partial loop fill injection

This example describes how to let the AS 100 transfer 360 µL from Reagent A to the destination vial, add 40 µL of sample and mix 3 times with 250 µL. In ‘Methods programming/Methods’ a flush volume of 50 µL is chosen.

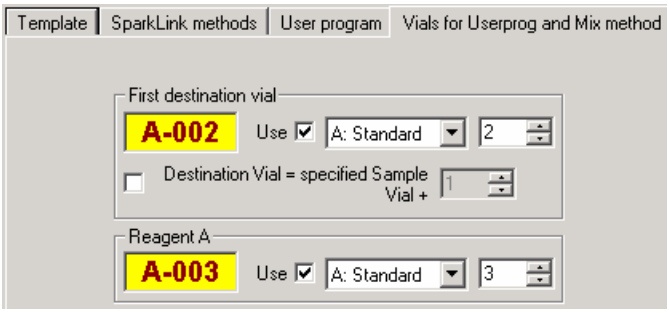


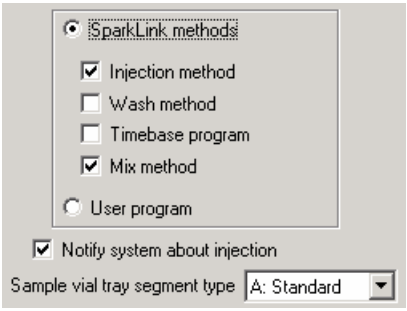
Click Add, edit, insert or delete to edit steps. Click ‘Set’ to add the programmed steps to the system method..

In ‘Methods programming/Methods’ the mix method is programmed. Note that aspirated volumes are a bit larger, therefore after dispense the remainder is removed to waste. Air segments are introduced to suppress dispersion of sample segments. Again aspirated volumes are a bit ‘oversized’. Mixing is done by repeated aspirating/dispensing steps.

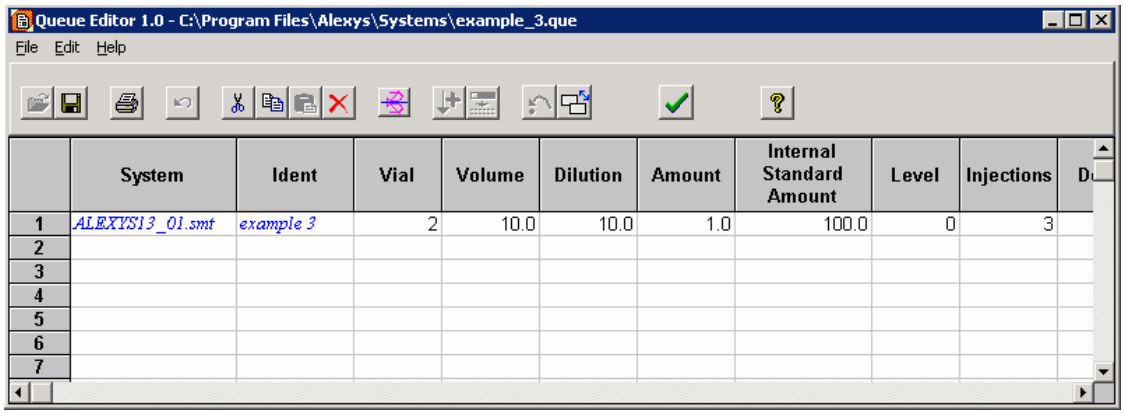
Program step	Description
001 Aspirate Air Volume - 0005 µL	aspirate an air segment of 5 µL
002 Aspirate from Reagent A Volume - 0225 µL	aspirate 225 µL from reagent vial A
003 Dispense to Destination Volume - 0180 µL	dispense 180 µL to destination vial
004 Dispense to Waste Volume - 0050 µL	dispense 50 µL to waste
005 Repeat steps - 04, times - 1	repeat last four steps once
006 Aspirate Air Volume - 0005 µL	aspirate an air segment of 5 µL
007 Aspirate from Sample Volume - 0060 µL	aspirate 60 µL of sample
008 Dispense to Destination Volume - 0040 µL	dispense 40 µL to destination vial
009 Dispense to Waste Volume - 0050 µL	dispense 50 µL to waste
010 Aspirate Air Volume - 0050 µL	aspirate an air segment of 50 µL
011 Aspirate from Destination Volume - 0200 µL	aspirate 200 µL from destination vial
012 Dispense to Destination Volume - 0200 µL	dispense 200 µL to destination vial
013 Repeat steps - 02, times - 3	repeat the last 2 steps three times
014 End	

Next step is to locate sample, destination and reagent vials in ‘Methods programming/Vials for Mix method’.





Set the correct checks in Template and put sample in position A 01. Position A 02 is used as empty destination vial. Place a filled reagent vial in position A 03. Make sure the reagent vial is filled before starting a new series. Program the queue editor for 3 injections of 10 µL from vial number 2.



	System	Ident	Vial	Volume	Dilution	Amount	Internal Standard Amount	Level	Injections	D
1	ALEXYS13_01.smt	example 3	2	10.0	10.0	1.0	100.0	0	3	
2										
3										
4										
5										
6										
7										

Note that mixing destination vial 2 is programmed for injection, vial 1 is the actual sample vial.

The AS 100 will now start searching for the Reagent vial and transport 180 µL to the destination vial twice, then 40 µL of sample will be added and after mixing 3 times a 10 µL injection will be performed.

C H A P T E R 5

Reference

This chapter describes all possibilities offered by the AS 100 software, in the order in which they appear in the screen when using a keypad. Same functionality (in fact even more) is in the ALEXYS data system software driver. For details see ALEXYS data system manual.

Ready Menu

The Ready Menu contains the following soft function keys:

<ADVANCE>
Use this key to rotate the tray of the AS 100. You can now fill the tray with segments by placing the segment at a free position in the front half of the tray.

<WASH>
Use this key to start a standard wash procedure. All tubing connected to the syringe valve will be filled and rinsed with wash solvent.

<SYR END>
Use this key to move the syringe to end position if you wish to replace the syringe needle or to simplify filling of wash solvent tubing. A syringe volume of wash solvent is aspirated from the wash solvent bottle and the wash solvent tube is filled.
Select soft function key <SYR HOME> to dispense the syringe contents to syringe waste and to move the syringe to standard operating position again.

<UTILS>
Use this key to go to the Utilities Menu. If use of a method protection code is enabled in the System Menu, the code must be entered to access the Utilities Menu. The menu offers the following possibilities:

<COPY> to copy a method. Enter the type (mix, injection, timebase, wash) and the number of the method to be copied. Then enter a number to define the destination method. Any existing method stored under that number will be overwritten.

<ERASE> to erase a method (Template, Methods, User Program). If Template and User Program are disabled in the System Menu, the soft function keys for erasing a standard Method (mix, injection, wash, timebase) appear. Note that it is not possible to erase the user program if the protection code for the user program is enabled in the System Menu.

<LOG> the AS 100 keeps a log of system-relevant events (<EVENTS>; records error messages that have been generated) and keeps count of actions of valves and syringe movements (<COUNT>). A message appears after every 50,000 syringe actions and after every 200,000 syringe valve actions: “Lifetime of syringe (valve) maybe exceeded. Check for possible leakage!”.
Syringe: if you do not replace the syringe at this moment and tell the system “not to display this message again”, the message will not be displayed again until 50,000 more syringe actions have been counted. **Syringe valve**: will have to be replaced and the counter for valve actions will have to be reset by the maintenance engineer.

<DEFAULT ALL> to change all software settings to default. All series, methods, templates and the user program (unless protected by protection code) will be erased.

If <DEFAULT ALL> is selected, check whether hardware configuration still is compatible with settings entered in the System Menu.

<SSV> (option)
Use this key to start a procedure in which all lines of the solvent selection valve can be primed. The menu offers the following possibilities:

<SSV1> to **<SSV6>** to open the corresponding port of the solvent selection valve.

<PRIME> to prime the selected solvent line with one syringe volume last selected port will remain active after leaving the SSV mode.

<COOL> (option)
Use this key to enter the programming mode for Peltier tray cooling. The programmable temperature range is 4°C to 40°C. The maximum cooling

capacity is approximately 20°C below ambient (refer to Appendix B for specifications). Connect the condensed water and leakage connector to a waste container on the floor to drain condensed water.
If the cool option is switched <ON> the following soft function keys can be selected:

<MANUAL>	temperature control will remain OFF until it is switched on again by the user (in this menu).
<AUTOMATIC>	temperature control will be switched OFF after all programmed series have been executed.
<DATE-TIME>	temperature control will be switched OFF at a date and time that can be programmed.

<SERIAL>
Use this key to put the AS 100 in serial mode to allow for control of the autosampler by way of PC (RS232 interface). Select a device identifier in the System Menu (refer to chapter 5). If a method protection code was defined in the system settings, this code must be entered to get access to serial mode. The following soft function keys appear:

<PANIC>	press this key to begin a stop sequence in which all tubing is rinsed and the valve and I/O ports are reset. At the end of the sequence serial mode is resumed.
<EXIT>	press this key to end serial mode and return to the Ready Menu.

<SERVICE>
For service to the apparatus. To be used by authorized personnel only. The Service Menu is protected by a service code.

System Menu

The System Menu contains the following soft function keys:

<GENERAL>
Press this key to enter values for:

- **loop volume, needle tubing, syringe volume**: these values have to be entered because the AS 100 can be fitted with various types of

syringes. Every needle and needle tubing volume requires a different minimum flush volume. The default flush volume equals two times the volume of needle and tubing.

The 10 mL syringe can only be selected if the use of the User Program is enabled in the System Menu (Usage Menu). When the 10 mL syringe is selected, only the User Program can be used. Use of other types of methods will be disabled.

- **syringe speed and scale factor:** The aspirating speed of the syringe used in injection methods can be adapted depending on viscosity of samples. Alternatively syringe speeds can be reduced by entering a scale factor. The syringe speed will be the scale factor multiplied by the syringe speed. The speed of the syringe during the wash or the rinsing procedure of the buffer are not affected by this setting. (Refer to table 5.3).

- needle height: distance between the needlepoint and a reference point, a few millimetres above the bottom of the sample tray (Not the bottom of the vial, see Fig. 24), can be programmed. The value in the system settings is only used in injection methods, for mix methods this value is programmable in the method itself).

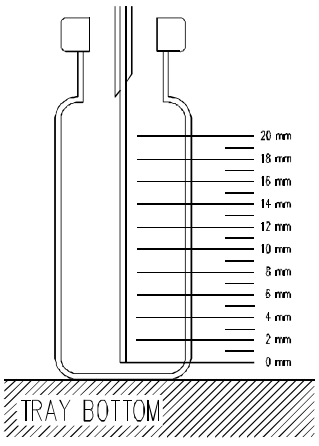


Fig. 24. Needle height.

A height of 0 mm is not the bottom of the tray, but a reference value related to a point a 2.5 mm above the bottom of the tray. Check sample needle height with an empty vial.

- **skip missing vials**: YES means that empty spaces are skipped during the run. NO means that the AS 100 will stop if an empty space is encountered during the run; an error code will be generated.
- **air segment**: decide whether an air segment will be used for analytical runs (for explanation of air segment refer to Appendix H).
- **headspace pressure**: to switch headspace pressure on or off. The AS 100 uses headspace pressure to facilitate transport of sample into the loop. The compressor will always be used during a wash procedure. Please note that accuracy and reproducibility may decrease if headspace pressure is switched off. However, headspace pressure will only be useful if sample vials are airtight (refer to chapter 2).
- **time display**: offers a choice between two types of time representation.
- **key click, error beep and alarm buzzer**: offer the possibility to switch sound signals on or off.

Table 5.1 Overview of General settings

general setting for	default	possible range
loop volume	100 µL	5 - 1000 µL
needle tubing	15 µL	1 - 200 µL
syringe volume	250 µL	100, 250, 500, 1000 µL or 10 mL
syringe speed	normal	low, normal or high
scale factor	1.0	0.1 - 1.0
needle height	2 mm	0 - 40 mm
skip missing vials	yes	yes or no
air segment	yes	yes or no
headspace pressure	yes	yes or no
time base display	HH:MM:SS	H:MM:SS or H:MM:mm
key click	on	on or off
error beep	on	on or off
alarm buzzer	on	on or off

<USAGE>

Press this key to enter the following Usage settings:

- **protection code**: to enter a code for protection of methods. Enter a six digit code (000000-999999) for protection of all methods. Press **CL** to erase the code. If a code has been defined it is not possible to enter the System Menu and the programming menus without entering the protection code. Default: none.
- **timebase methods**: to enable or disable the possibility to program timebase methods. The AS 100 controls other connected equipment during analysis time. Program timebase methods in the Methods Menu. Default: disabled.
- **mix methods**: to enable or disable the possibility to program mix methods for the AS 100. Program mix methods in the Methods Menu. Please note that the AS 100 cannot analyse priority samples during a run if the mix method is enabled. Default: disabled.
- **user program**: to enable or disable the possibility to program a user program. If this function is enabled it is possible to enter a user program protection code (6 digits). Program the user program in the Methods Menu. Please note that the AS 100 cannot analyse priority samples during a run if the user program is enabled.
  Default: disabled.
- **labelled vials**: to enable or disable the possibility to program labelled vials. Program the location of labelled vials in the Series Menu.
  Default: disabled.
- **templates**: to enable or disable the possibility to program templates. Program templates in the Methods Menu. Default: disabled.
- **calibration vials**: to enable or disable the possibility of programming calibration vials. Program the location of the calibration vials in the Series Menu. Default: disabled.

You are advised to disable as many functions in the Usage Menu as possible to make sure that other menus do not contain possibilities that are irrelevant for the type of analyses you are to perform.

<TRAY>

Use this key to define the type of tray that will be used. Four types of tray segments can be selected: Type A (default), type B, type C and type D

numbered 1-8 (see Fig. 9). After all tray types have been has been selected, enter:

- **type of transport vials (A-D),** or press **CL**
- **first transport vial**: enter a number
- **last transport vial**: enter a number

Vials can be placed in any of the vial positions. Transport vials must be placed in a continuous row.

<IO>

Use this key to enter the I/O configuration mode and define the following:

- **vial output BCD or HEX**: to define the vial output.
- **inject-marker pulse length**: to define the length of the inject-marker pulse.
- **vial-marker pulse length**: to define the length of the vial-marker pulse.
- **labelled vial marker pulse length**: to define the length of the vial-marker pulse of the labelled vial.
- **input edge next injection**: to define the edge sensitive inputs for the next injection.
- **input edge next vial**: to define the edge sensitive inputs for the next vial.
- **freeze input active**: to define whether the freeze input is active when high, or freeze input is active when low.
- **reset outputs after last series**: to determine whether the outputs should be reset to default after the last series.

Refer to Chapter 6 for more specific information on I/O connections.

Table 5.2 Overview I/O menu

Possibility	default	Range
inject-marker pulse length	1.0 s	0.1 - 2.0
vial-marker pulse length	1.0 s	0.1 - 2.0
labelled vial marker pulse length	1.0 s	0.1 - 2.0
input edge next injection	falling	falling or rising
input edge next vial	falling	falling or rising
freeze input active	low	low or high
reset outputs after last series	no	yes or no

<CLOCK>
Use this key to switch the system clock on or off. Select ON to enter the clock menu in which you can set date (yy,mm,dd) and time (hh,mm). This date and time will be displayed in the Ready Menu.

<COMM.>
Use this key to define a device identifier for communication with other equipment (e.g. a PC). An identifier between 20 and 29 can be selected for the AS 100.

Methods Menu

This menu allows the user to program various types of methods: it is possible to define 24 separate injection methods, 5 wash methods, 5 timebase methods, 9 mix methods and one user program.
It is also possible to program a combination of methods and save them in a **template**. The settings entered in the System Menu determine the possibilities offered by the Methods Menu.

<TEMPLATE>
Use this key to enter a menu in which the contents of a template can be defined. First assign a number to the template, then link the numbers of methods to the template. The following items can be entered to fill a template:

- **user program instead of methods**: if soft function key <YES> is selected, the complete template is filled with the user program; no other methods can be added. If soft function key <NO> is selected the template can be filled with the following:
- **mix method number**
- **injection method number**
- **wash method number**
- **timebase method number.**

A maximum of 24 templates can be programmed.

<METHODS>
Use this key to enter a menu in which methods can be defined:

<MIX> (if enabled in System Menu, Usage Menu)
Use this key to program a method that allows you to perform pre-injection sample handling, e.g. pre-column derivatisation, dilution or adding of internal standard. Nine mix methods can be programmed; the maximum number of steps that can be programmed for the total of 9 mix methods and the user program is 240. Assign a number to the mix method. The Mix Menu appears:

- <EDIT> an existing step or a new step for a new mix method
- <INSERT> a new step in an existing method before the displayed step
- <DELETE> the displayed step.

“End of mix method” means that the mix method is empty; if an existing mix method is selected, the first line of the mix method is displayed. Scroll through the steps of the existing method with the cursor keys and use the soft function keys to enter changes in an existing method.
The following types of steps can be programmed for a mix method:

<ASPIRATE> (sample, air, destination, reagent A-D) a programmed volume. Speed of syringe can be selected from 1-9. (Refer to table 5.1, page 34 for values). Height (H) indicated is the distance of the needle point to the tray holder (default: 2 mm).The maximum amount which can be aspirated is the total volume of the syringe.

<DISPENSE> (sample, waste, destination, reagent A-D) a programmed volume from the buffer tubing. Speed of the syringe can be selected from 1 - 9. (Refer to table 5.1, page 34). Height (H) indicated is the distance of the needle point to the sample tray (default: 2 mm). It is possible to dispense a larger volume than the volume aspirated in previous actions. The aspirated amount will be complemented with liquid from the wash solvent bottle to total the programmed dispense volume.

<WAIT> to define a pause (H:MM:SS, maximum of 9 hours, 59 minutes and 59 seconds).

During the pause, the needle will move to home position (if the previous step is an aspirate or dispense action). If you want the needle to stay in the same position, an aspirate or dispense step of 0 µL must be programmed at the desired position.

<REPEAT> Enter the number of steps that must be repeated and how often they must be repeated.

<WASH> enter the volume for needle wash. Buffer is rinsed to waste.

<INJECTION>

Use this key to program a method that defines injection methods (max. 24) for a run of the types full loop, partial loop fill or µL pick-up. Enter a number for the injection method you are going to program. If the selected method is locked because of changes in the settings (System Menu) after programming the method, the word LOCK is displayed. The method can be unlocked by programming valid values in the method itself or by restoring the values in the System Menu.
Use the soft function keys to select an injection method, then enter values for:

- **analysis time**: the time between switching the injection valve to inject and the start of processing the next sample.
- **flush volume**: the amount of sample taken from a vial before the loop is filled with sample. Default value: 30 µL (combined with an air segment).

Flush volumes of less than twice the volume of the needle and tubing will result in decreased performance.

- **number of injections per vial:** maximum value is 9.
- **injection volume:** can be entered for each injection per vial. The maximum programmable injection volumes are:

  partial loop fill: 50% of the programmed loop volume
  µL pick-up: injection volume = (loop volume – 3 x needle volume)/2
  flushed loop: not programmable, is equal to the loop volume but needs more sample to fill the loop (3 x loop volume for loop volumes < 100 µL; 2 x loop volume for loop volumes ≥ 100 µL - 499 µL; 1.5 x loop volume for loop volumes ≥ 500 µL).

<WASH>

Use this key to program wash methods. It is possible to program a wash between injections, samples or series. For each wash method the volume

of wash solvent can be defined. The minimum programmable volume is 300 µL.

<TIMEBASE> (if enabled in System Menu, Usage Menu)
Press this key to enable control of the optional ISS valve and other devices via auxiliary or binary outputs. A maximum of 5 timebase methods can be programmed. The menu offers the following soft function keys:

<AUX> scroll through all program lines by pressing **E** or select AUX to move to the next auxiliary.

<VALVES> controls the ISS valve and the solvent selection valve. The ISS valve can only be programmed if the optional ISS valve is installed. 6-1 and 2-1 refer to the interconnected ports of the valves. Press **E** to scroll through programming lines (Only if SSV option is installed). Enter the time and the SSV port number (value between 1 and 6).

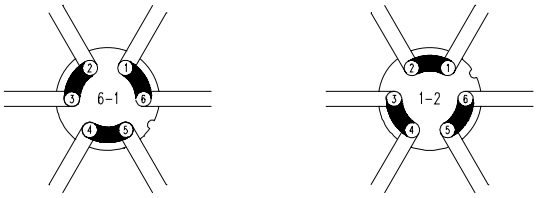


Fig. 25. ISS valve; interconnected ports

<CODE> outputs: enter a time and a value between1 and 15, hexadecimal output. Press **E** to scroll through the programming lines.

<END> to enter the end time for timed events program; press **E** to scroll through the programming lines. If no value is filled in or if **CL** is pressed, the AS 100 will automatically generate an end time. The end time is equal to the analysis time programmed in the injection method used in the same series.

If end time exceeds the programmed analysis time, this end time overrules the analysis time. It is possible to program events after the end time, but these events are not carried out during a run.

<USER PROGRAM>

The user program offers the possibility to program all possible actions required for a sample handling sequence in separate steps. Note that the total number of steps for the user program and all nine mix methods cannot exceed 240. The user program can be protected by a special user program protection code (System Settings, Usage Menu). If no user program has been programmed yet, “end of user program” is displayed. Otherwise, the first line of the programmed method appears. The soft function keys appear:

- <EDIT> an existing step or program a new step for the user program
- <INSERT> a new step before the displayed step
- <DELETE> delete the displayed step.

The edit and insert menus offer the following soft function keys:

<ASPIRATE	a programmed volume from sample vial, ambient air, destination vial, wash, or one of the reagent vials into the buffer tubing. Speed and height of syringe can be entered (refer to table 5.1). The maximum volume that can be aspirated is the total volume of the syringe.
<DISPENSE>	a programmed volume from the buffer tubing into the sample vial, waste, destination vial, wash or one of the reagent vials. Speed and height of syringe can be entered (refer to table 5.1). It is not possible to dispense a larger volume than the total volume aspirated in previous actions.
<SYR_VALVE>	to control the connections of the syringe to one of its three tubes: <NEEDLE>: connection to sample needle <WASH>: connection to wash solvent bottle <WASTE>: connection to syringe waste tubing.
<SYR>	to control the movements of the syringe. <LOAD>: the syringe with the programmed volume <UNLOAD>: the syringe with the programmed volume <HOME>: the volume previously aspirated will

be dispensed to the last programmed position, and the syringe will be initialised again.

<WASH> to execute a needle wash; the content of the buffer tubing is not rinsed to waste before the start of the wash. The programmed volume of wash solvent is used to wash the needle at the wash position.

The wash position may be contaminated with the contents of the buffer tubing, which may generate cross-contamination. To prevent contamination of the wash position, program a dispense-to-waste action before programming a wash action.

<VALVES> to program positions of high pressure valves (ISS, injector valve, SSV). The injector valve has two positions: <INJECT> and <LOAD>. The ISS optional valve has positions 1-6 and 1-2 (see Fig. 25).

<WAIT> to program a pause (max. 9 hours, 59 minutes, 59 seconds).

During the pause, the needle will move to home position (if the previous step is an aspirate or dispense action). If you want the needle to stay in the same position, an aspirate or dispense step of 0 µL must be programmed at the desired position.

<COMPRES> to activate the compressor to put air pressure on a sample. The compressor will stay active until it is switched off (in a next programmed step). The compressor will be automatically switched off at the end of the needle wash routine if a needle wash is used.

<AUX> to control the four standard auxiliaries (contact closures). Refer to Chapter 6.

<WAIT-IN> to program a pause in which the AS 100 waits for one of the four inputs to become <HIGH> or <LOW> before continuing with the next step. Refer to Chapter 6.

<PROG-OUT> to define two programmable outputs (contact closures). These are similar to the auxiliaries, but only available in the user program. Refer to Chapter 6.

<CODE> to program the output to the connector P3 TIMED OUTPUTS. This is a HEX output in the range 0 to 15. Refer to Chapter 6.

<MARKERS> the markers normally generated in the AS 100 are not active in the user program, but can be programmed in this screen (refer to Chapter 6). Select marker and status (inject, vial, labelled).

<SSV> (option): to define the Solvent Selection Valve (SSV) port position, range 1 to 6.

Table 5.3 Syringe speed

100 uL Syringe

	Flow in mL/min. through needle					
Scale	**Load**			**Unload**		
Factor	**Low**	**Normal**	**High**	**Low**	**Normal**	**High**
0,1	0,01	0,03	0,04	0,05	0,11	0,14
0,2	0,03	0,05	0,08	0,11	0,21	0,27
0,3	0,04	0,08	0,11	0,16	0,32	0,41
0,4	0,05	0,10	0,15	0,22	0,43	0,55
0,5	0,06	0,12	0,19	0,27	0,53	0,69
0,6	0,08	0,15	0,22	0,33	0,64	0,83
0,7	0,09	0,17	0,26	0,38	0,74	0,96
0,8	0,10	0,20	0,30	0,44	0,87	1,13
0,9	0,11	0,22	0,34	0,49	0,96	1,28
1	0,12	0,25	0,38	0,55	1,07	1,37

250 uL Syringe

	Flow in mL/min. through needle					
Scale	**Load**			**Unload**		
Factor	**Low**	**Normal**	**High**	**Low**	**Normal**	**High**
0,1	0,03	0,06	0,09	0,14	0,27	0,34
0,2	0,06	0,13	0,19	0,27	0,53	0,69
0,3	0,09	0,19	0,28	0,41	0,80	1,02
0,4	0,13	0,25	0,37	0,55	1,07	1,37
0,5	0,16	0,31	0,47	0,69	1,33	1,71
0,6	0,19	0,37	0,56	0,81	1,60	2,09
0,7	0,22	0,44	0,65	0,96	1,85	2,40
0,8	0,25	0,49	0,75	1,09	2,18	2,82
0,9	0,28	0,56	0,84	1,23	2,40	3,20
1	0,31	0,62	0,94	1,37	2,67	3,43

500 uL Syringe

	Flow in mL/min. through needle					
Scale	Load			Unload		
Factor	Low	Normal	High	Low	Normal	High
0,1	0,06	0,13	0,19	0,27	0,53	0,68
0,2	0,13	0,25	0,38	0,55	1,06	1,37
0,3	0,19	0,38	0,56	0,82	1,60	2,04
0,4	0,25	0,50	0,74	1,09	2,13	2,74
0,5	0,31	0,62	0,93	1,37	2,67	3,43
0,6	0,38	0,74	1,12	1,63	3,20	4,17
0,7	0,44	0,87	1,30	1,92	3,69	4,80
0,8	0,50	0,99	1,50	2,18	4,36	5,65
0,9	0,56	1,12	1,68	2,46	4,80	6,40
1	0,62	1,25	1,88	2,74	5,33	6,86

1000 uL Syringe

	Flow in mL/min. through needle					
Scale	Load			Unload		
Factor	Low	Normal	High	Low	Normal	High
0,1	0,13	0,25	0,38	0,55	1,06	1,36
0,2	0,25	0,50	0,75	1,09	2,11	2,74
0,3	0,38	0,75	1,12	1,64	3,20	4,09
0,4	0,50	1,00	1,49	2,18	4,27	5,49
0,5	0,63	1,25	1,87	2,74	5,33	6,86
0,6	0,75	1,49	2,23	3,25	6,40	8,35
0,7	0,87	1,75	2,59	3,84	7,38	9,60
0,8	1,00	1,98	3,00	4,36	8,73	11,29
0,9	1,12	2,23	3,37	4,92	9,60	12,80
1	1,25	2,49	3,77	5,49	10,67	13,71

During the dispense action the pressure in the buffer tubing will increase. To prevent damage of the buffer tubing, the flow should not exceed the value of 6 mL/min for water. (Maximum speed 9 for 100 µL and 250 µL-syringes, speed 6 for a 500 µL syringe and speed 4 for 1000 µL and 10 mL syringe.) If more viscous liquids are used the speeds should be reduced.

Series Menu

This menu allows you to define the run sequence in a series. A maximum of 24 series can be programmed. A series contains information about the methods to be used for a range of vials. This can be a template, a separate method (mix, injection, wash, timebase), or the user program. Information on location of vials, labelled vials or calibration vials is also programmed in a series.

Table 5.4 Series parameters

Without templates	***With templates***
# Use user program Yes/No * Injection method number * Wash method number #Time base methods number * Mix method number *Time base and mix method are only available if enabled in the System Menu*	* Template number
# Use calibration vials Yes/No # First calibration vial # Last calibration vial # No. of samples between calibration *Calibration vials are only available if enabled in the System Menu; not available if Mix Method has been programmed*	
* First sample vial * Last sample vial	
Only if a mix method has been programmed, or if used in user program: # First destination vial # Vial Reagent-A # Vial Reagent-B # Vial Reagent-C # Vial Reagent-D	
Only if the use of labelled vials has been enabled in the System Menu: # Labelled vial no. 1 # Labelled vial no. 2 # Labelled vial no. 3 # Labelled vial no. 4	

* marked questions are always asked in series,
# marked questions depend on the used methods and the settings entered in the System Menu.

The settings entered in the System Menu and the methods defined in the Methods Menu determine which possibilities appear in the Series Menu.

Explanation:
After you have entered the required settings in the System Menu and after you have programmed methods to be used for an analytical run, you can press **Series** to enter the Series Menu. Table 5.4 gives an overview of the items you have to define for the Series.

- With Templates
  If you are going to execute an analytical run by way of a template, you will only be asked to enter the template number and to indicate the location of the first sample vial and the last sample vial.

- Without Templates
  If you are going to execute an analytical run without using a template, you will be asked to enter an injection method number and a wash method number, and you will have to indicate the location of the first sample vial and last sample vial.

If you have enabled use of calibration vials in the System Menu (Usage Menu), you will have to define whether you will use calibration vials, and indicate the location of the first and last calibration vial, and indicate the number of vials between calibration vials (refer to Fig. 26).
However, if you have for example enabled use of a Mix Method in the System Menu (Usage Menu), you will also have to define the location of the First destination vial and Reagent vials.

Series are stored in the AS 100 memory for as long as the power is on. As soon as power is switched off, all programmed series will be deleted.
It is not possible to leave the Series Menu before all values have been programmed.

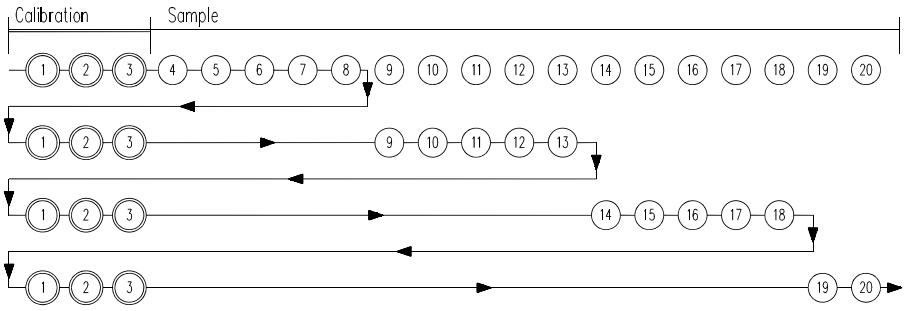


Fig. 26. Injection sequence with 3 calibration vials between every 5 vials

C H A P T E R 6

I/O Connections

The AS 100 has six standard I/O connectors on the rear side; five OUTPUT connectors and one INPUT connector. Refer to Fig. 2 for location of connectors.

The communication connector is a standard RS232 or an RS422/485 communication interface connector. The configuration of the I/O connector is described in the tables below.

The manufacturer will not accept any liability for damages directly or indirectly caused by connecting the AS 100 to instruments, which do not meet relevant safety standards.

Contact closures outputs

The following three tables with programmable outputs (P1), marker outputs (P4) and auxiliary outputs (P5) are contact closures outputs (floating NO/NC contact); see Fig. 27.

Table 6.1 Connector P1 OUTPUTS (2 programmable outputs and alarm output)

1	OUT 1 - Normally open
2	OUT 1 - Common
3	OUT 1 - Normally closed
4	OUT 2 - Normally open
5	OUT 2 - Common
6	OUT 2 - Normally closed
7	Spare
8	Spare
9	Spare
10	Alarm output – Normally open
11	Alarm output – Common
12	Alarm output - Normally closed
13	24 V DC
14	Power ground
15	Power ground

VMAX = 28 VDC / VAC, IMAX = 0.25 A

The Alarm output will be activated whenever an error occurs; refer to Appendix C for a description of the error codes of the AS 100.

Table 6.2 Connector P4 MARKERS

1.	Inject marker - Normally open
2.	Inject marker – Common
3.	Inject marker - Normally closed
4.	Vial marker - Normally open
5.	Vial marker – Common
6.	Vial marker - Normally closed
7.	Labelled vial marker - Normally open
8.	Labelled vial marker - Common
9.	Labelled vial marker - Normally closed
10.	STOP I/O - Normally open
11.	STOP I/O - Common
12.	STOP I/O - Normally closed
13.	24 V DC
14.	Power ground
15.	Power ground

V_{MAX} = 28 V_{DC} / V_{AC}, I_{MAX} = 0.25 A

Table 6.3 Connector P5 AUXILIARIES

1.	AUX 1 - Normally open
2.	AUX 1 - Common
3.	AUX 1 - Normally closed
4.	AUX 2 - Normally open
5.	AUX 2 - Common
6.	AUX 2 - Normally closed
7.	AUX 3 - Normally open
8.	AUX 3 - Common
9.	AUX 3 - Normally closed
10.	AUX 4 - Normally open
11.	AUX 4 - Common
12.	AUX 4 - Normally closed
13.	24 V DC
14.	Power ground
15.	Power ground

V_{MAX} = 28 V_{DC} / V_{AC}, I_{MAX} = 0.25 A

Maximum current for 24 V_{DC} supply is 0.5 A total.

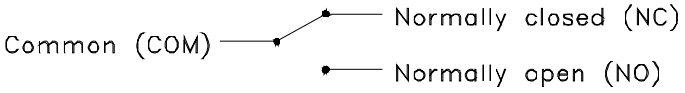


Fig. 27. Contact closures outputs

TTL outputs

The following tables show the marker outputs (P2) and a 4-bit time base code output (P3), programmable in a time base method. Both connectors are TTL level outputs; a connection diagram is shown in Fig. 28.

Table 6.4 Connector P2 TTL OUTPUTS

1.	INJECT MARKER.
2.	VIAL/VIAL MARKER.
3.	LABELLED VIAL MARKER.
4.	STOP I/O.
5.	D O (BCD or HEX) (1).
6.	D 1 (BCD or HEX) (2).
7.	D 2 (BCD or HEX) (3).
8.	D 3 (BCD or HEX) (4)
9.	D 4 (BCD or HEX) (10 or 16)
10.	D 5 (BCD or HEX) (20 or 32)
11.	D 6 (BCD or HEX) (40 or 64)
12.	D 7 (BCD or HEX) (80 or 128)
13.	Signal ground
14.	Signal ground
15.	Signal ground

All markers are active low (logical 0). V_{MAX} = 5.5 V, logical 1 > 3.5 V, logical 0 < 1.0 V. DC output source / sink current ± 20 mA.

A marker output pulse will be generated when the injection valve switches from LOAD to INJECT. However, in a User Program markers have to be programmed by the user.

BCD: Binary Coded Decimal

The BCD output consists of a 2-digit vial number. D3-D0 represent the single digits (0-9) and D7-D4 represent the tens.

Table 6.5 BCD output conversion

Value • = active	Output D 0 (1)	 D 1 (2)	 D 2 (4)	 D 3 (8)
0				
1	•			
2		•		
3	•	•		
4			•	
5	•		•	
6		•	•	
7	•	•	•	
8				•
9	•			•

Table 6.6 Examples of BCD vial number output

BCD	Tens				Single digit				Vial number
	D7	D6	D5	D4	D3	D2	D1	D0	
	(8)	(4)	(2)	(1)	(8)	(4)	(2)	(1)	
Output	1	0	0	1	0	1	1	0	96 90 + 6
	9 x 10 (10^1)				6 x 1 (10°)				
Output	0	1	0	1	1	0	0	1	59 50 + 9
	5 x 10 ($10^{1)}$				9 x 1 (10°)				
Output	0	0	0	1	0	0	0	0	10 10 + 0
	1 x 10 (10^1)				0 x 1 (10°)				

Hex: Hexadecimal Code

The hexadecimal output represents the vial number as 8 bits by assigning values of 1, 2, 4, 8, 16, 32, 64 and 128 to every single bit (D0-D7).

Table 6.7 Examples of hexadecimal output

HEX	D7 (128)	D6 (64)	D5 (32)	D4 (16)	D3 (8)	D2 (4)	D1 (2)	D0 (1)	Vial number
Output	1	0	0	1	0	1	1	0	**150**
	128 + 16 + 4 + 2								
Output	0	1	1	0	0	0	0	0	**96**
	64 + 32								
Output	0	1	0	1	0	1	0	1	**85**
	64 + 16 + 4 + 1								
Output	0	0	1	1	1	0	1	1	**59**
	32+ 16 + 8 + 2 + 1								

Table 6.8 Connector P3 TIMED OUTPUTS; 4-bit time base code output

1 TB 0 (HEX) (1)	6 Signal ground
2 TB 1 (HEX) (2)	7 Signal ground
3 TB 2 (HEX) (4)	8 Signal ground
4 TB 3 (HEX) (8)	9 Signal ground
5 not used	

V_{MAX} = 5.5 V, logical 1 > 3.5 V , logical 0 < 1.0 V. DC output source / sink current ± 20 mA.

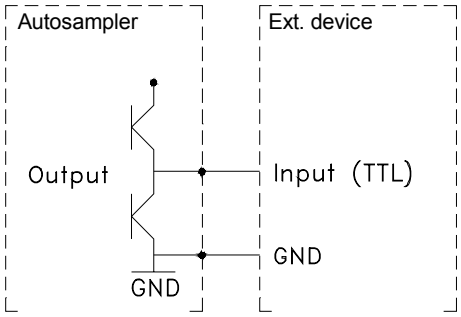


Fig. 28. TTL output

TTL inputs

The following connector is an active high or active low TTL input; it can be defined in the System Menu. The NEXT INJECTION INPUT and the NEXT VIAL INPUT can be used when the AS 100 works in REMOTE CONTROL. The FREEZE INPUT and STOP I/O input can be used to control the AS 100 by other devices. The four inputs (INPUT 1 to 4) can only be used in the user program, e.g. to control the sequence of the steps in this method. A connection diagram is shown in Fig. 29.

Next injection input:
This input will start the next injection sequence when the AS 100 is started in remote control. When the injection sequence is finished the AS 100 will wait for the next input.
From the Ready Menu a NEXT INJECTION INPUT will start the last programmed series. In this case the AS 100 will not wait for the NEXT INJECTION INPUT before continuing with the next injection. The AS 100 will execute the complete RUN as if it was started with the **Start/Stop** key.

Table 6.9 Connector P6 INPUTS (TTL)

1	NEXT INJECTION INPUT	9	Signal ground
2	NEXT VIAL INPUT	10	Signal ground
3	FREEZE INPUT	11	Signal ground
4	STOP I/O	12	Signal ground
5	INPUT 1	13	Signal ground
6	INPUT 2	14	Signal ground
7	INPUT 3	15	Signal ground
8	INPUT 4		

Next vial input:
With this input the AS 100 will perform the next injection from the next vial, even if not all injections from that vial in the programmed injection method have been executed.

Freeze input:
The AS 100 will freeze the analysis time for the time this input is active. If the FREEZE INPUT is activated while the analysis time is not running, the AS 100 will perform all programmed pre-injection sample handling (mix method and loading part of the injection method). But the AS 100 will wait with injecting the sample until the FREEZE INPUT is no longer active.

Stop I/O:
With this input the run of the AS 100 is immediately aborted. The Ready Menu appears in the display. In case the AS 100 is in remote control, the run of the AS 100 is immediately aborted but the AS 100 remains in remote control and cannot be restarted with a NEXT INJECTION INPUT.

INPUT 1-4:
Programmable input, can be used in the user program.

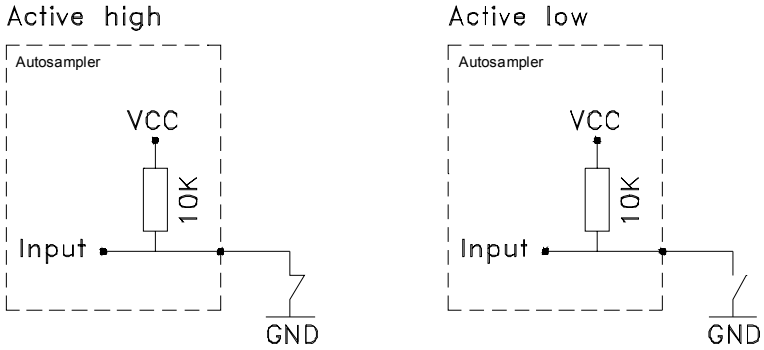


Fig. 29. TTL input

MultiLink connector

The MultiLink connector is used to make the AS 100 communicate with computer software.

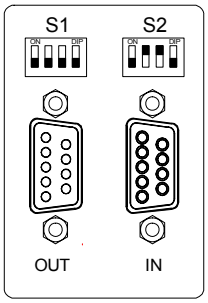
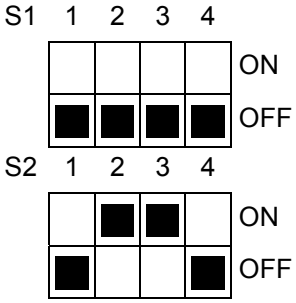


Fig. 30. MultiLink board

Set the dipswitch S1 and S2 as follows to let the communication work probably:



Port		Description
S1:	OUT	to next device
S2:	IN	to PC or previous device

Connections for 9-pin connector:

pin 2	TD	Transmitted data to the computer.
pin 3	RD	Received data from the computer.
pin 5	SG	Signal ground (Also indicated as GND in some devices)

A P P E N D I X A

Installation

The AS 100 shipping container is supplied with a packing list. Please check that all items mentioned in the list are included in the package before you start the installation procedure for the AS 100.

Do not install the AS 100 in areas subject to shock, dust, or in direct sunlight. Do not place it near a source of heat, as this will disturb tray cooling (if this option is installed).

The AS 100 must only be connected to power sources and apparatus with protective grounding.

Fig. 31. Connectors AS 100, D - A - P order: detector, autosampler and pump.

Execute the following steps to install the AS 100:

1. Lift the AS 100 from the shipping container. Make sure you keep the apparatus upright; lift it by placing your hands under the AS 100.
2. Place the AS 100 in its operating location between OR 100 and DECADE II as shown in chapter ‘Introduction’. Make sure the ventilation holes are not obstructed. Allow the instrument to acclimatize for 1 hour.
3. Connect serial cable as shown in Fig. 31.

4. Install tray (Fig. 1) in the AS 100.
5. Check whether local voltage matches voltage indicated on back panel of the AS 100. Change if necessary (including fuses).
6. Connect the power cord of the AS 100 (Fig. 2 [5]) to one of the OR 100 power outlets (Fig. 31).
7. Switch the AS 100 on by using the switch at the back of the apparatus (Fig. 2 [4]).

The AS 100 now starts up. If present, the display indicates that a self-test and initialisation procedure are executed. After completion of this procedure the following appears on the display:

10:26 TUESDAY DECEMBER 16-04 [MENU] READY (x.xx)
<ADVANCE> <WASH> <SYR END> <UTILS>

Factory installed items

The AS 100 is factory-installed with:

- fuses in power switch:
  115 V (AC) ± 10%: two 5 AT fuses (slow, ¼" x 1¼", UL/CSA) or
  230 V (AC) ± 10%: two 2.5 AT fuses (slow, 5 x 20 mm, IEC127).
- 100 µL loop
- 250 µL syringe.
- 500 µL buffer tubing
- stainless steel 15 µL sample needle

The fuses used are UL-listed and CSA-certified.

Tubing	**Material and dimensions**
Standard sample needle and tubing	SS tubing; 135 mm x 0.65 mm O.D. x 0.25 mm I.D. Tefzel tubing; 140 mm x $^{1}/_{16}$" O.D. x 0.25 mm I.D. (total volume 15 µL)
Buffer tubing from high pressure valve to syringe valve	PTFE tubing; 640 mm x $^{1}/_{16}$" O.D. x 1.0 mm I.D. (volume 500 µL)
Tubing syringe valve to wash solvent bottle	PTFE tubing; 300 mm x $^{1}/_{16}$" O.D. x 1.0 mm I.D.
Tubing syringe valve to waste	PTFE tubing; 400 mm x $^{1}/_{8}$" O.D. x 1.6 mm I.D.

For installation of HPLC connections, waste tubing, wash solvent, syringe, sample needle, trays, etc. refer to Chapter 2 of this manual.
Installation of software driver is described in the user manual of ALEXYS data system.

A P P E N D I X B

Specifications ALEXYS AS 100 Autosampler

General specifications	Power	115 or 230 VAC ± 10 %, 50/60 Hz, 250 VA max
	Fuses	115 VAC: two 5.0 AT (¼" x ¼", UL/CSA)
		230 VAC; two 2.5 AT (5 x 20 mm, IEC 127)
		All fuses UL-listed and CSA-certified
	Internal fuse	One 6.3 AT fuse (5 x 20 mm, IEC 127)
	Operating conditions	10 – 35°C, 20 – 80% RH, non-condensing
	Storage	-25 to +60°C
	Sample capacity	160, 96, 72 or 32 vials (20, 12, 9 or 4 per segment)
	Vial dimensions	7, 12, 15, or 22 mm vial diameter min/max height: 32/46 mm (including cap) micro vial inserts for micro dialysis
	Dispenser	syringe: 100, 250 (standard), 500, 1000 µL, or 10 mL
	Wetted parts	SS316, PTFE, TEFZEL, VESPEL, glass, PEEK, silica
	Loop volume	5 µL – 1 mL (1, 2, 5 steps), 100 µL standard
	Precautions	pre-puncturing septa/caps with air needle, dual needle action, vial & height detection by vial sensor
Analytical performance	Flushed loop	RSD ≤ 0.3 % (all specs for capped and sealed vials)
	Partial loop fill	RSD ≤ 0.5 %, injection volumes ≥ 5 µL, with headspace pressure on the vial and 30 µL pre-flush with air segment
	µL Pick-up	RSD ≤ 1.0 %, injection volumes ≥ 5 µL, with headspace pressure on the vial
	Memory effect	< 0.01 % with programmable needle wash
Injection programming	Methods	flushed loop, partial loop and µL pick-up
	Volumes	1 µL – 1 mL (method depended)
	Injections	vial, volume, repetitions: freely programmable in ALEXYS data system queue editor
	Wash	programmable needle wash
Connections	Outputs	marker TTL and contact closures: inject, vial, labelled vial, stop I/O; programmable: 4 TTL, 4 relays contacts no/nc, 2 relays contacts no/nc, 4 TTL time based; alarm
	Inputs	TTL: next injection, next vial, freeze, stop, 4 TTL
	PC control	RS232C, full control with ALEXYS data system

Options	Sample cooling	4 - 40°C; accuracy: ± 2° C capacity: ambient - 20° C (measured on cooling tray)
	Micro option	optimised for small injection volumes ≥ 1 µL; with 5.3 µL needle, 20 µL loop and 100 µL syringe volume, µ-bore valve
Physical specifications	Dimensions	54 (D) x 28 (W) x 44 (H) cm
	Weight	22 kg, 30 kg incl. cooling option

A P P E N D I X C

Error Codes

The AS 100 will display an **error message** if the user tries to enter invalid data. Information on the allowed range will be displayed. If something goes wrong in the physical operations of the AS 100, an **error code** will be displayed. Press the **Start/Stop** key twice to lift the message and try to repair the failure condition with the help of the explanation of the code concerned.

Injection valve and ISS unit

ERROR 11	Injection valve is not in a valid position.
ERROR 12	The injection valve did not switch within 1.5 seconds.
ERROR 13	The switching time of the injection valve exceeds 500 msec.
ERROR 14	ISS A valve is not in a valid position.
ERROR 15	The ISS A valve did not switch within 1.5 seconds.
ERROR 17	ISS valve B is not in a valid position.
ERROR 18	ISS valve B did not switch within 1,5 seconds.

Syringe dispenser unit

ERROR 21	The syringe valve did not switch.
ERROR 22	The syringe did not reach home position in time.
ERROR 23	The syringe spindle did not make the correct number of rotations.
ERROR 24	The spindle does not rotate.
ERROR 25	The syringe valve did not find a valid position.

Injection needle unit

ERROR 30	The sample needle arm did not reach or leave home position (vertical).
ERROR 31	The sample needle arm is in an invalid horizontal position while moving down.
ERROR 32	The sample needle arm did not reach or leave destination within a certain time (horizontal).
ERROR 33	The sample needle arm needed too many or too few steps to reach destination.

ERROR 34	Sample needle arm not in vertical home position while moving horizontally.
ERROR 35	The sample needle is at an invalid horizontal position.
ERROR 36	The sample needle detects too many code gaps while moving to the next position.
ERROR 37	The sample tray is not at a valid position while moving down the needles. Stop sensor is not in tray gap.
ERROR 39	Vial sensor sticks.
ERROR 40	The sample needle spindle does not rotate correctly.
ERROR 41	The sample needle did not reach or leave home position.
ERROR 42	The sample needle is not at home position.

Tray

ERROR 51	Incorrect tray rotation.
ERROR 52	No segment found.
ERROR 53	The sample needle arm is not in the home position while moving the tray.

Vials

ERROR 60	Missing vial. Only available when Skip Missing Vial is set to NO in the System Settings.
ERROR 61	Missing segment.(Only available when Skip Missing Vial is set to NO in the System Settings and during the execution of a Mix Method).
ERROR 62	Missing transport vial.
ERROR 63	Missing transport segment. (Only available when Skip Missing Vial is set to NO in the System Settings and during the execution of a Mix Method).
ERROR 64	Missing vial for reagent A.
ERROR 65	Missing vial for reagent B.
ERROR 66	Missing vial for reagent C.
ERROR 67	Missing vial for reagent D.
ERROR 68	Missing destination vial.
ERROR 69	Not enough transport liquid available due to missing transport vials or segments.

Electronics

ERROR 71	Flex print of the sample needle is not connected.
ERROR 72	Invalid configuration of the AS 100, PCB missing.
ERROR 73	Current limit of the external I/O exceeded.
ERROR 75	Error occurred during initialisation, the AS 100 cannot start.

A P P E N D I X D

Options and accessories

The following configuration options are available for the AS 100:

Part no.	Description
	needles, valves and syringes
181.0306	Needle holder
181.0510	Needle holder assembly
181.0508	Needle unit assembly
181.0304	Needle wash insert vial
181.0310	Plunger replacement tip 100 µL (pck/10)
181.0542	Plunger replacement tip 250µL (pck/10)
181.0342	Syringe 100 µL luerlock
181.0344	Syringe 250 µL
181.0506	Syringe dispenser assembly
181.0328	Syringe valve
181.0320	Syringe waste tubing, extra long
181.0346	Valco 100µL PEEK loop
181.0348	Valco 100µL Stainless steel loop
181.0370	Valco 20µL Stainless steel loop
181.0356	Valco ship kit for C2-2006
181.0324	Valco Stainless steel valve C2-2006 SPHT, .4 mm
181.0362	Valco micro stainless steel valve C2-1006 SPHT, .25mm
181.0326	Valco Peek valve C2-2346 SPHT
181.0316	Peek sample needle 15µL valco
181.0312	St. steel sample needle 15µL valco
181.0366	Fused silica sample needle 5.3 uL valco
181.0322	Air/prepuncturing needle
	sample trays
	note: s=segment, p=position
181.0600	Tray set cool; 8 s, 1.8 mL, 12 p, 12 mm
181.0602	Tray set (std); 8 s, 1.8 mL, 12 p, 12 mm
181.0604	Tray set 0.5 mL, type D.
181.0606	Tray set 4 mL, type B.
181.0608	Tray set 10 mL, type C.
181.0610	Tray segment; standard tray, number 1
181.0612	Tray segment; standard tray, number 2
181.0614	Tray segment; standard tray, number 3
181.0616	Tray segment; standard tray, number 4
181.0618	Tray segment; standard tray, number 5
181.0620	Tray segment; standard tray, number 6

181.0622 Tray segment; standard tray, number 7
181.0624 Tray segment; standard tray, number 8
181.0626 Tray segment; cooling option, number 1
181.0628 Tray segment; cooling option, number 2
181.0630 Tray segment; cooling option, number 3
181.0632 Tray segment; cooling option, number 4
181.0634 Tray segment; cooling option, number 5
181.0636 Tray segment; cooling option, number 6
181.0638 Tray segment; cooling option, number 7
181.0640 Tray segment; cooling option, number 8
181.0672 Tray segment 4mL Type B number 1
181.0688 Tray segment 10mL Type C number 1
181.0704 Tray segment 0.5mL Type D number 1

parts and spares

181.0200 AS 100 ship kit
181.0010 AS 100 user manual
181.0318 Buffer tubing 500 µL
181.0552 Code sensor with brush
181.0578 Fuse 2.5 AT
181.0594 Fuse 5 AT
181.0588 Inject marker cable
181.0576 Optical sensor
181.0666 Outer shipping box only
181.0368 Rotor seal micro for Valco C2-1006
181.0332 Rotor seal for Valco C2-2006
181.0334 Rotor seal for Valco C2-2346
181.0544 Rubber feet selfadhesive
181.0596 Serial cable 9m-9f, 3m
181.0358 Silicon tubing 7.0-10mm
181.0546 Stop sensor assembly
181.0354 T-connector waste tubing
181.0308 Transport Nut M5
181.0302 Tube connector
181.0314 Tubingset 900
181.0512 Wash position assembly
181.0360 Wash solvent assembly
181.0340 Wash solvent bottle 250 mL

A P P E N D I X E

Calibration and performance

The AS 100 is factory tested for reproducibility, carry-over and mixing according to the following test procedure.

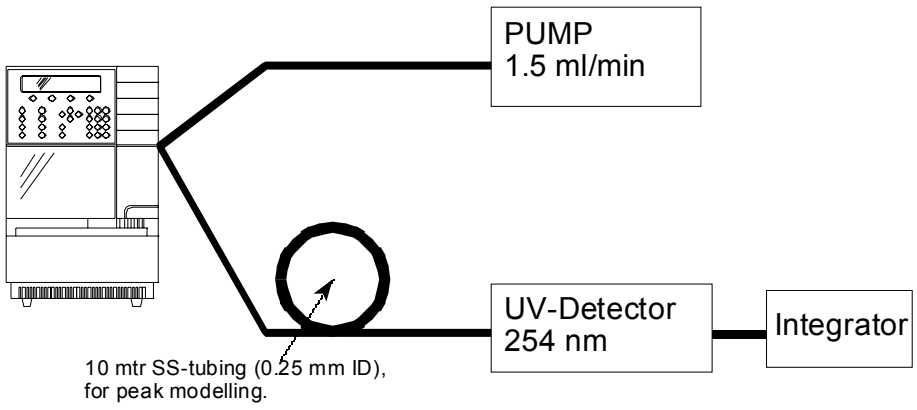
Analytical performance test

Analytical system

The AS 100 is tested in an analytical system under the following conditions:

Pump	flow:	1.5 mL/min
	eluent:	distilled water
Detector	wavelength:	254 nm
Sample:		Uracil in distilled water (50 ppm)

The following configuration was used:



Relative standard deviation (RSD%)

The following formulas are required for calculating the RSD:

$$\overline{Peak\ area} = \frac{\sum Peak\ area}{n}$$

$$\sigma_{n-1} = \sqrt{\frac{\sum\left(Peak\ area - \overline{Peak\ area}\right)^2}{n-1}}$$

$$RSD\% = \frac{\sigma_{n-1}}{\overline{Peak\ area}} \times 100\%$$

Reproducibility test

The default system settings were used, except for the following:

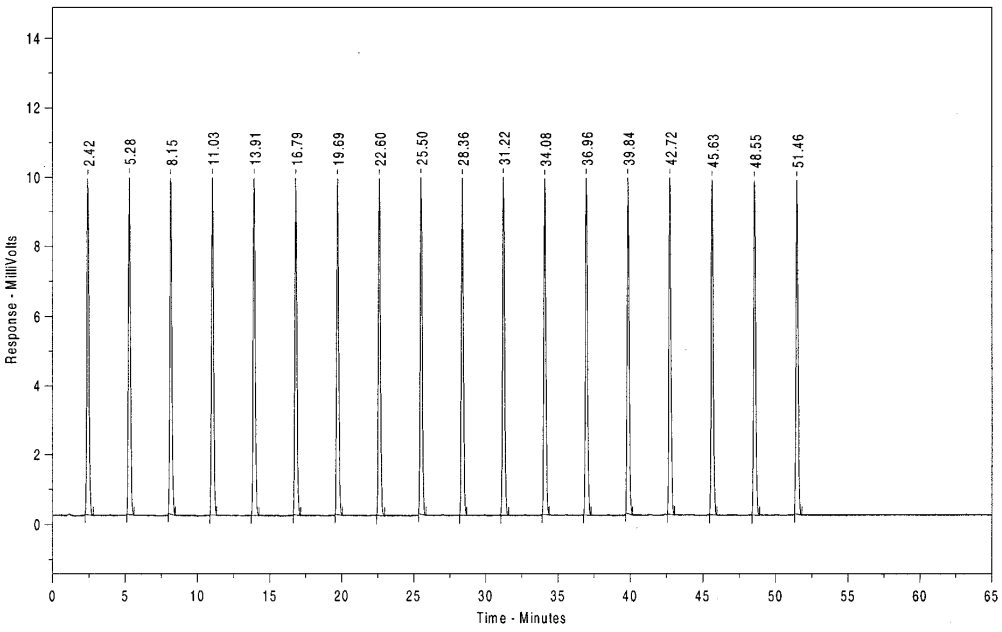
<GENERAL>	Air segment: NO
<TRAYS>	Location first transport vial: A7
	Location last transport vial: A7

Sx vials are filled with sample on positions A1 - A6.
The transport solvent vial is filled with eluent and placed on position A7.

The wash solvent bottle is filled with 80% H_2O / 20% isopropanol.

Injection method		**Wash method**		**Series**	
Number	1	Number	1	Number	1
Type	µL pick-up	Wash between injections		First vial	A 01
Analysis time	1:00 min	Wash volume	300 µL	Last vial	A 06
Injections/vial	3			Injection method	1
Injection volume 1	5 µL			Wash method	1
Injection volume 2	5 µL				
Injection volume 3	5 µL				

Example chromatogram of the reproducibility test:



Peak number	Area
1	206342
2	204773
3	205425
4	205203
5	205566
6	205568
7	206156
8	206174
9	205956
10	206165
11	205765
12	205960
13	206296
14	205617
15	205619
16	205648
17	206147
18	207070

From the integration results the RSD% for the AS 100 can be calculated.

For this example the results are:

RSD% = 0.25 %

The specified value is an RSD% < 1%.

Mixing and carry-over test

The default system settings were used, except for the following:

<USAGE> Use of mix methods: ENABLED

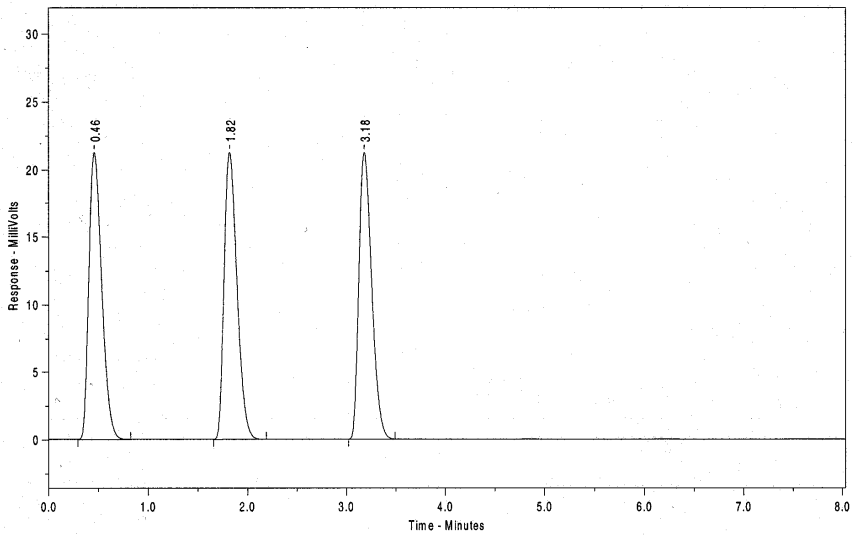
A tray with one sample vial filled with sample (50 ppm Uracil, A01), one sample vial filled with eluent (A02), and one empty (destination, A03) vial are placed in the AS 100. A 1:10 dilution of sample from the sample vial will be made in the empty destination vial. After dilution from both vials (sample and destination) 10 µL will be injected. The Reagent-A vial is filled with eluent.
The wash solvent bottle is filled with 80% H_2O / 20% isopropanol.

Injection method		**Series 1**		**Series 2**	
Number	1	Number	1	Number	2
Type	partial loop fill	Injection method	1	Injection method	1
Analysis time	1:00 min	Wash method	None	Wash method	none
Flush volume	35 µL	Mix method	1	Mix method	none
Injections/vial	3	First vial	A 01	First vial	A 01
Injection volume 1	10 µL	Last vial	A 01	Last vial	A 02
Injection volume 2	10 µL	First destination vial	A 03		
Injection volume 3	10 µL	Reagent A vial	A 02		

Mix method 1

Step	**Action**			**Speed**	**Height**
1	Aspirate	5 µL	Air	2	
2	Aspirate	225 µL	Reagent A	2	02
3	Dispense	180 µL	to Destination	3	02
4	Dispense	50 µL	to Waste	5	
5	Repeat last 4 steps 1 time				
6	Aspirate	5 µL	Air	2	
7	Aspirate	60 µL	Sample	2	02
8	Dispense	40 µL	to Destination	3	02
9	Dispense	50 µL	to Waste	5	
10	Aspirate	50 µL	Air	2	
11	Aspirate	200 µL	from Destination	3	0
12	Dispense	200 µL	to Destination	9	20
13	Repeat last 2 steps 3 times				
14	End of mix method				

Example chromatogram of the mixing and carry-over test:



Peak number	Area
1	38802
2	38968
3	39088
4	395258
5	394090
6	394471
7	-
8	-
9	-

From the integration results the RSD% for the two vials and the carry-over for the AS 100 can be calculated.

For this example the results are:
RSD% (vial A01, sample) = 0.15 %
RSD% (vial A03, 1:10 dilution) = 0.37 %

1:10 dilution = 1:10.13: variation -1.29%
The specified variation is ± 5%

Carry-over (vial A02): < 0.2%
The real value cannot been calculated, the specified value is a Carry-over <0.2%.

Syringe calibration

To calibrate the syringe dispense a volume of 200 µL water from a sample vial to an empty destination vial with the following mix method.

Mix method 1

Step	Action			Speed	Height
1	Aspirate	250 µL	Sample	2	02
2	Dispense	200 µL	to Destination	3	02
3	End of mix method				

Series 1	
Injection method	None
Wash method	None
Mix method	1
First sample vial	A 01
Last sample vial	A 01
First destination vial	A 02

    - Weigh the destination vial before and after the Run.
    - The difference is the dispensed volume
- The specified variation is ± 2%

Tray cooling calibration

1. Place a thermocouple on the bottom of the tray; make sure the contact is good.
2. Switch on the tray cooling and program a set point of 10°C
3. Wait minimal 15 minutes for equilibration of the AS 100
4. Read out the temperature of the thermocouple.

The value must be in a range of ± 2°C of the programmed set point.

Loop calibration

1. Disconnect the loop from the injection valve.
2. Remove all liquids from the loop with air.
3. Weigh the empty loop on an analytical balance.
4. Fill the loop with minimal 2 times its volume of water.
5. Weight the filled loop again.
6. The difference in weight is the capacity of the loop.
7. The weight divided by the specific weight of water (1 g/mL) gives the calibrated volume of the loop.

Allowed variation: ± 10% (According to Rheodyne).

A P P E N D I X F

Maintenance injection valve

The AS 100 is standard equipped with a Valco C2-2006/2346 injection valve. Cleaning a valve can often be accomplished by flushing all lines with appropriate solvents. With normal use the valve will give many tens of thousands of cycles without trouble. The main cause of early failure, which is seen as a leak in the valve, is abrasive particles in the sample and/or mobile phase, which can scratch the rotor seal. Following is the procedure for changing the rotor seal.

NOTE:
Do not disassemble the valve unless system malfunction is definitely isolated to the valve.

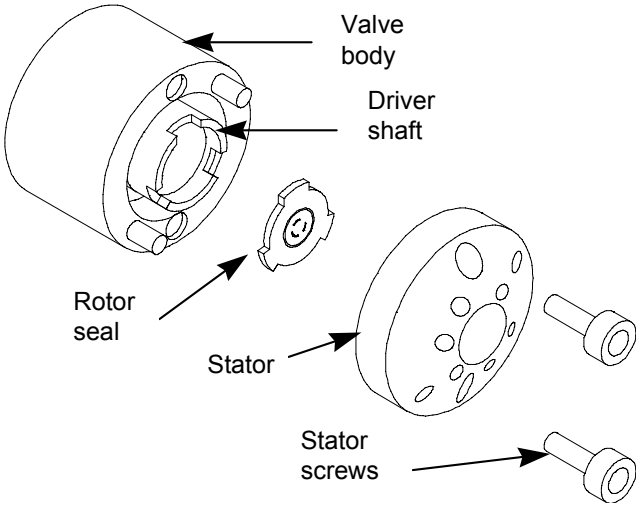


Fig. 32. Exploded view of injection valve.

Disassembly

1. Use a 9/16 hex driver to remove the socket head screws which secure the cap on the valve.
2. To insure that the sealing surface of the cap is not damaged, rest it on the outer face. Or, if the tubing is still connected, leave it suspended by the tubing.
3. With your fingers or small tool, gently pry the rotor away from the driver.

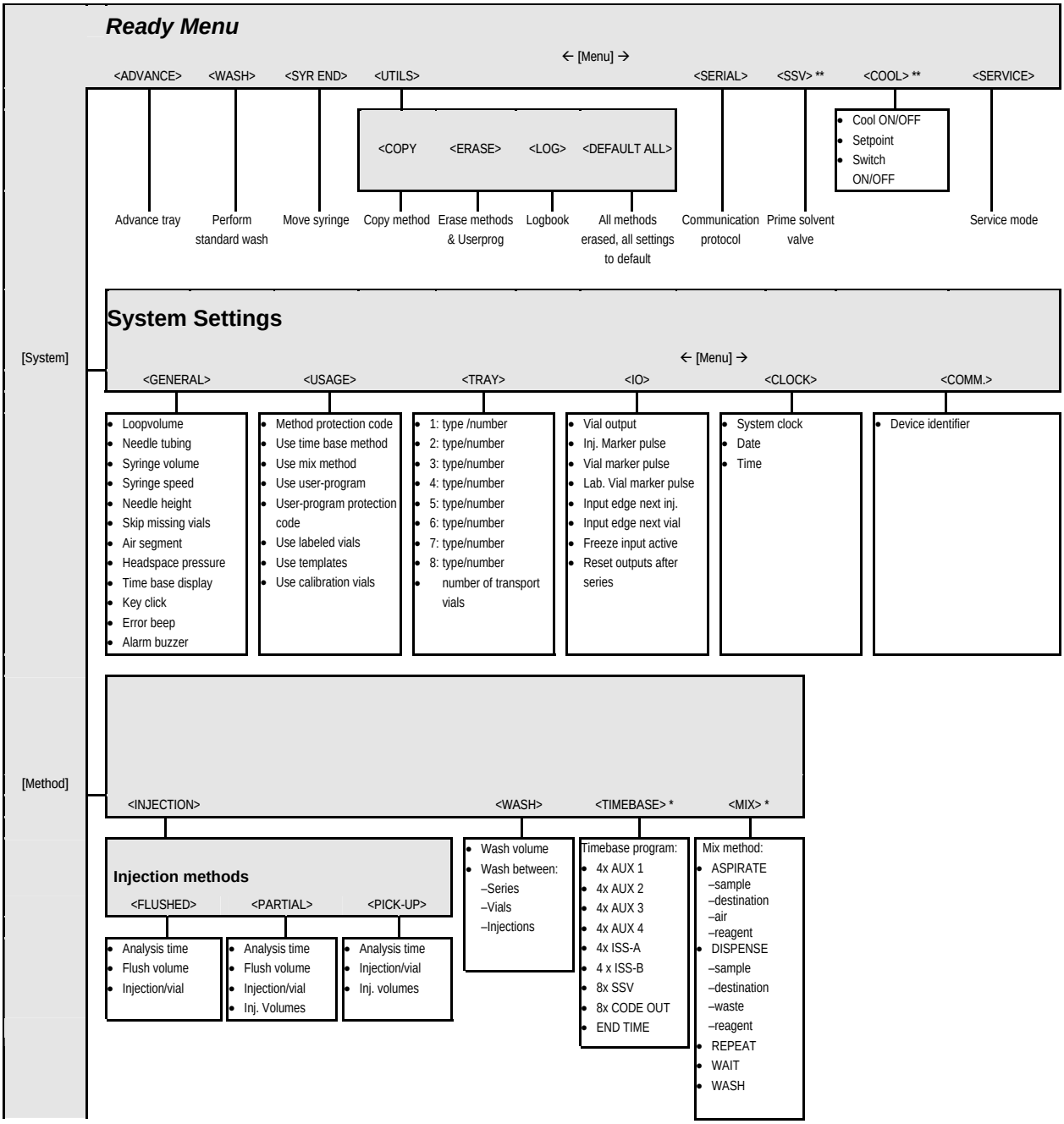
4. Examine the rotor sealing surface for scratches. If scratches are visible to the naked eye, the rotor must be replaced. If no scratches are visible, clean all the parts thoroughly with an appropriate solvent, taking care that no surfaces get scratched. (The most common problem in HPLC is the formation of buffer crystals, which are usually water-soluble) It is not necessary to dry the rotor.

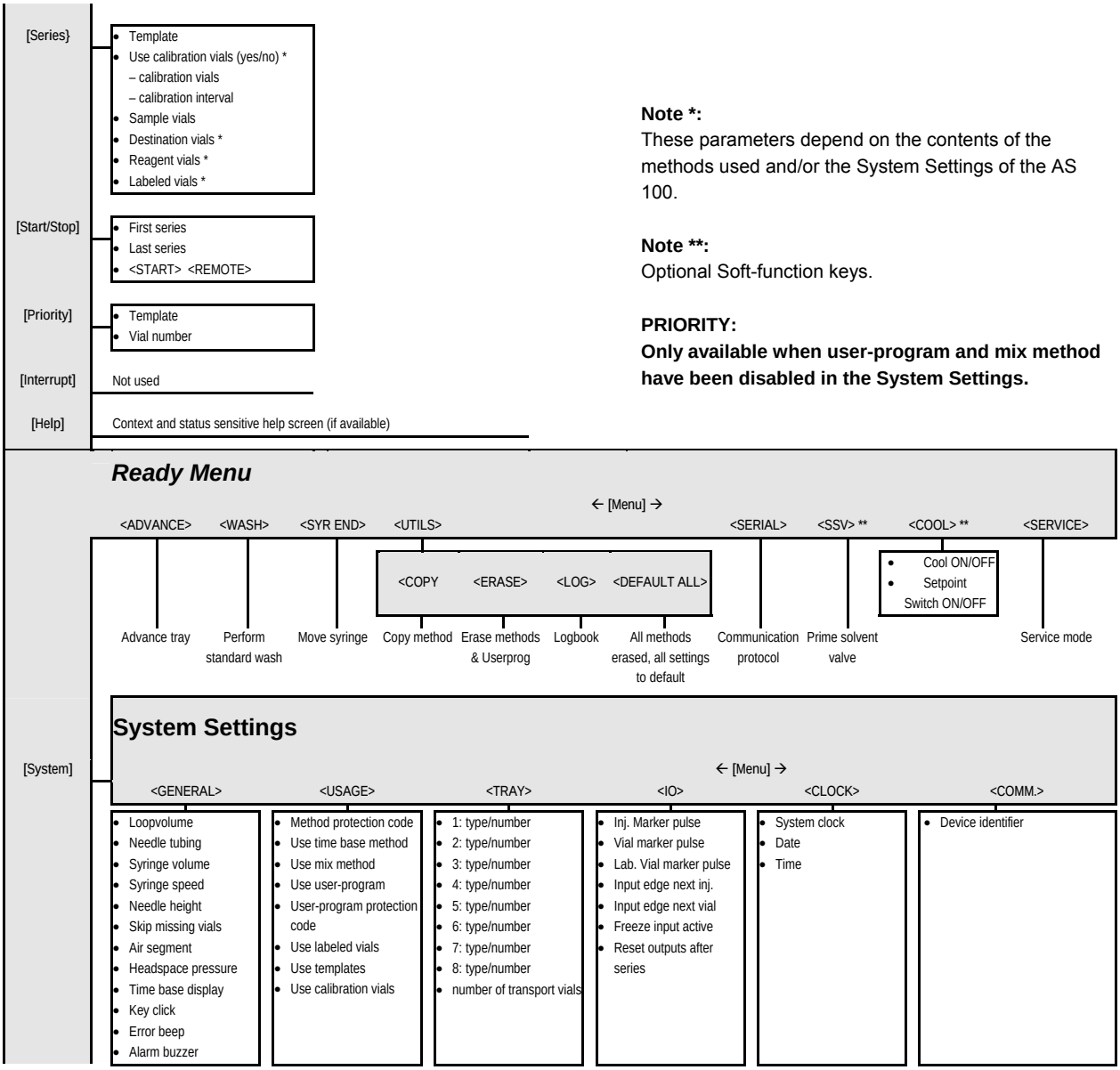
Reassembly

1. Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out. The pattern is asymmetrical to prevent improper placement.
2. Replace the cap. Insert the two socket head screws and tighten them gently until both are snug. *Do not over-tighten them* - the screws simply hold the assembly together and do not affect sealing force, which is automatically set as the screws close the cap against the valve body.
3. Test the valve by pressurizing the system. If it does not hold pressure, the valve should be returned to Valco for repair.

A P P E N D I X G

Programming options

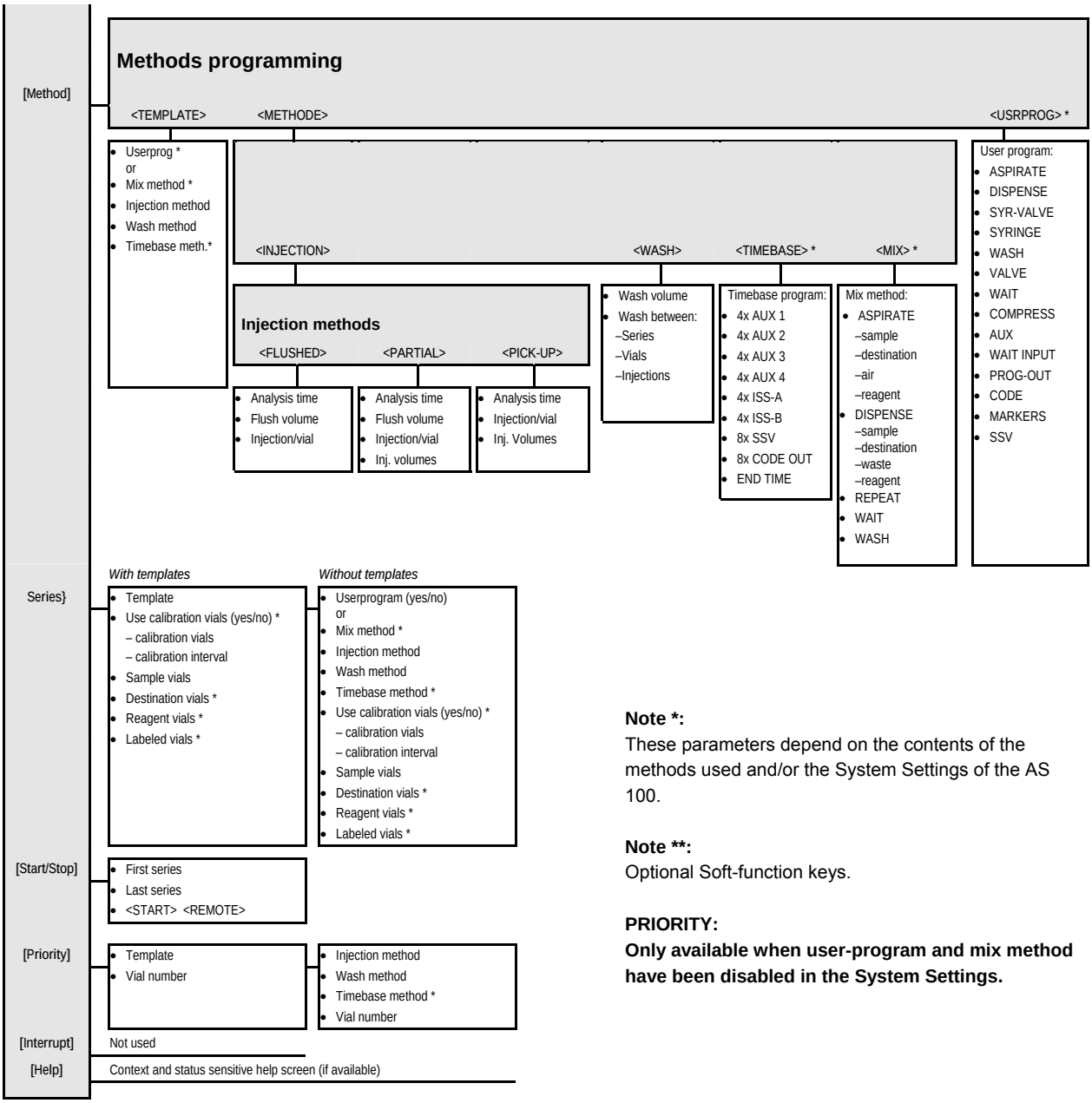




Note *:
These parameters depend on the contents of the methods used and/or the System Settings of the AS 100.

Note **:
Optional Soft-function keys.

PRIORITY:
Only available when user-program and mix method have been disabled in the System Settings.



Note *:
These parameters depend on the contents of the methods used and/or the System Settings of the AS 100.

Note **:
Optional Soft-function keys.

PRIORITY:
Only available when user-program and mix method have been disabled in the System Settings.

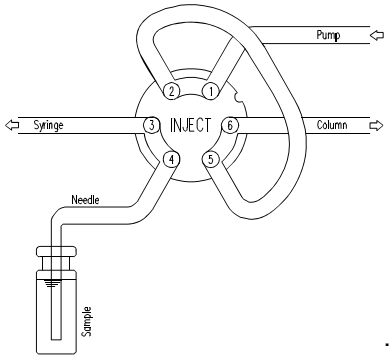
A P P E N D I X H

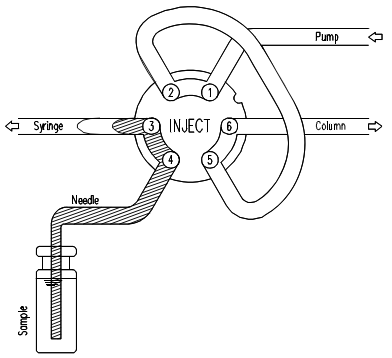
Injection principle

This appendix offers specialist information on the injection principles used by the AS 100.

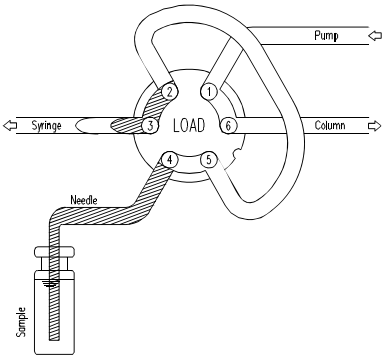
Flushed loop injections

The switching sequence for a flushed loop injection is schematically shown in the following figures:
The initial situation: the injector is in the INJECT position. The sample needle has entered the vial after the air needle has pre-punctured the septum. Headspace pressure is applied through the outer air needle to ensure that no air or vapour bubbles are formed during sample aspiration

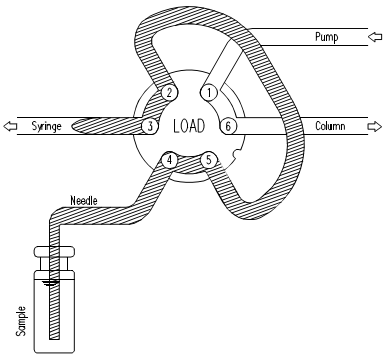




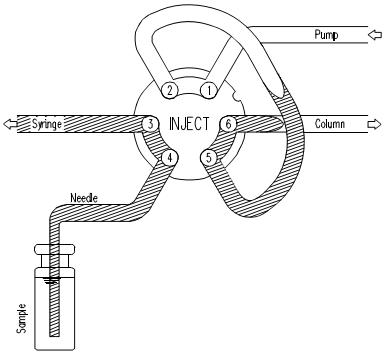
The syringe dispenser aspirates the programmed flush volume from the sample vial to fill the sample line with sample and remove wash solvents.



The injection valve switches in LOAD position, placing a "sharp" sample front at the inlet of the sample loop.



For flushed loop injections the sample loop is quantitatively filled by transporting two or more times the loop volume through the loop, depending on the volume of the loop.



The injection valve switches in INJECT position. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis time starts.

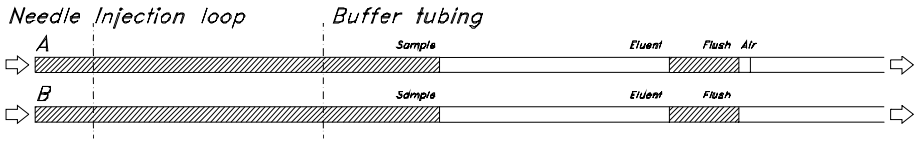
If one injection is to be done from each vial or if a wash routine has to be performed after every injection, the needle withdraws from the vial directly after the injection and, if programmed, directly performs a wash. After the analysis time a new sequence is started.

If more than one injection is done from the same vial without a wash routine, the AS 100 withdraws a flush volume after the analysis time to compensate for diffusion of mobile phase from the rotor groove into the first part of the sample line during the analysis time. The flush volume between injections is not programmable and is always 50% of the programmed flush volume. If the total amount of sample withdrawn with the next injection from the vial will exceed the total volume of the buffer tubing, the buffer tubing is emptied into the wash position before the next injection. The next fill sequence will then start with a full flush volume.

Air segment

An air segment can be used to reduce the amount of flush volume. This air segment is at the front of the flush volume and will not be injected and therefore will not influence the injection. Use of an air segment can be enabled in the System Menu (General Menu).

With a standard needle the flush volumes must be: minimal 30 µL for injections with air segment and 35 µL for injections without air segment. If the samples are highly viscous it may be necessary to program larger flush volumes and reduce the syringe speed for better performance.

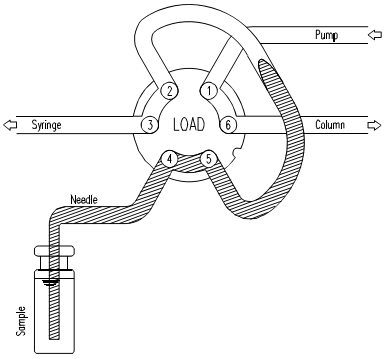


A = with air segment; B = without air segment

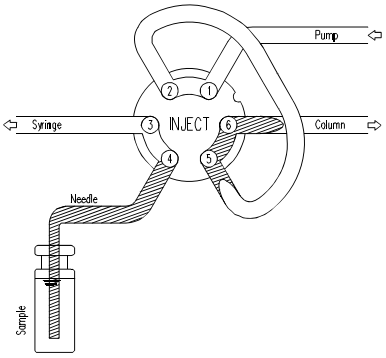
Partial loop fill injections

The switching sequence for a partial loop fill injection is schematically shown in the following figures:

The first three steps are identical to those for Full loop injections (see H.1).



For partial loop fill injections the sample loop is filled by transporting the programmed injection volume into the sample loop.

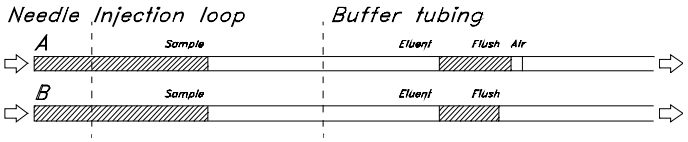


The injection valve switches into the INJECT position. The sample loop is now part of the HPLC mobile phase flow path: the sample is transported to the column. The analysis time starts.

The next injection sequence will start with a flush of 50% of the programmed flush volume, in case an injection from the same vial and no wash routine is programmed. Otherwise it will start with a flush of the programmed flush volume. If the aspiration of sample for the next injection

will exceed the total volume of the sample buffer tubing, the buffer tubing is emptied before the next injection. The next injection will start with the programmed flush, see also the full loop injections.

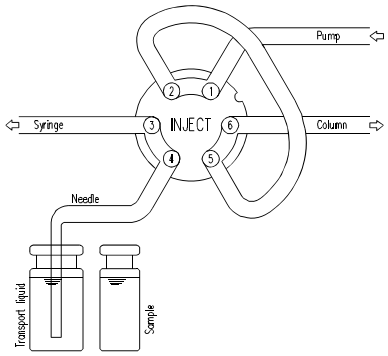
Air segment
An air segment can be used to reduce the amount of flush volume. This air segment is at the front of the flush volume and will not be injected. Use of an air segment can be enabled in the System Menu (General Menu).



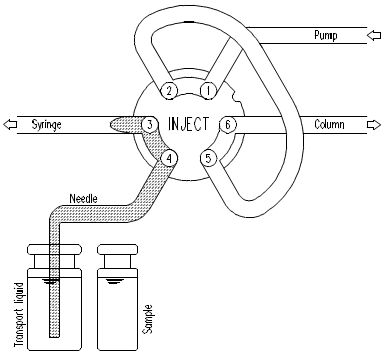
A = with air segment; B = without air segment

µL pick-up injections

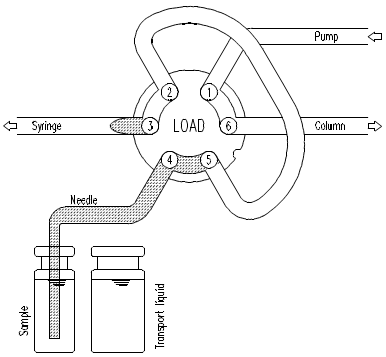
The switching sequence for a µL pick-up injection is schematically shown in the following figures:



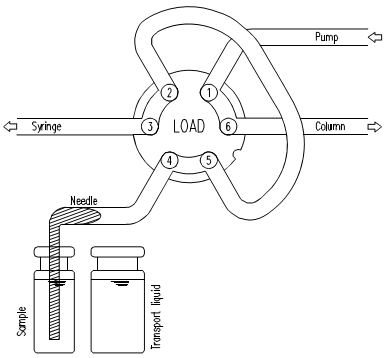
The initial situation: the injection valve is in INJECT position. The sample needle has entered the vial of transport liquid (mobile phase, to avoid disturbance of the chromatogram with an additional peak of the transport solvent) after the air needle has pre-punctured the septum. The headspace pressure, applied through the outer air needle, ensures that no air or vapour bubbles are formed during wash solvent aspiration.



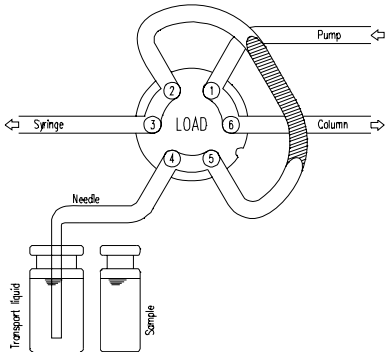
For the first injection after a wash or after emptying of the buffer tubing, the syringe dispenser aspirates transport liquid from the transport vial to fill the sample line with transport liquid and remove wash solvent.



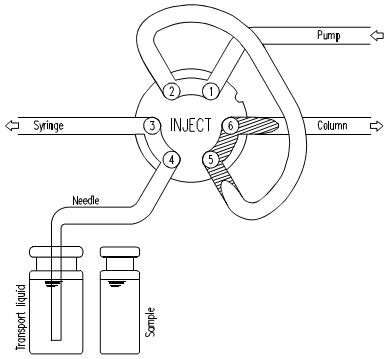
The needle moves from the transport vial to the sample vial. The injection valve is switched to the LOAD position.



The programmed injection volume is aspirated from the sample vial.



The sample needle moves back to the transport vial. The sample is quantitatively transported into the loop, with transport liquid (mobile phase) from the transport vial.



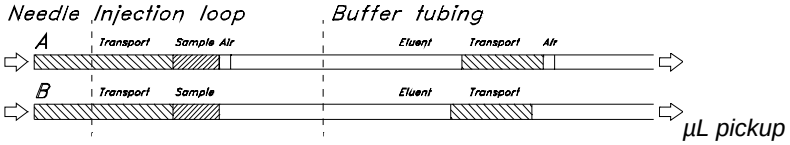
The injection valve is switched to INJECT. The sample loop is now part of the HPLC mobile phase flow path: sample is transported to the column. The analysis time starts to run.

The next sequence will skip the first withdrawal of transport solvent, unless a wash routine is performed or the AS 100 has emptied the buffer tubing to waste. In those cases the sequence is completely repeated.

Air segment

If an air segment has been programmed, it appears at the front of the first plug of transport liquid and at the front of every sample plug. Use of an air segment can be enabled in the System Menu (General Menu).

Note: The air segment at the front of the sample plug is injected into the HPLC system.

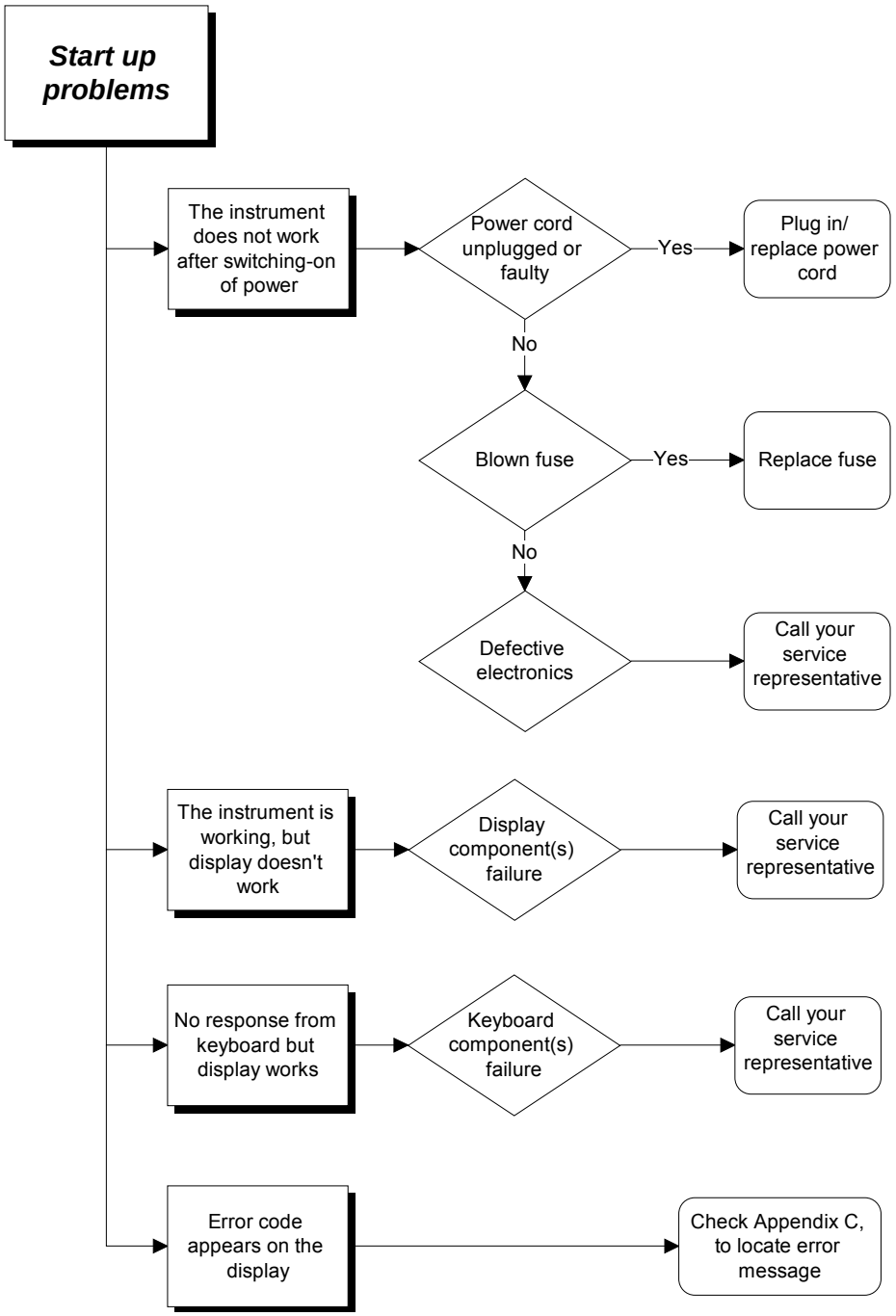


injections with (A) or without (B) air segment.

In case of µL pick-up injections there will be no air pressure (headspace pressure) on the sample vial to prevent errors due to air expansion during switching from sample vial to transport vial.
You are advised to switch off use of an Air segment in the System Menu if the µL pick-up injection method is used.

A P P E N D I X I

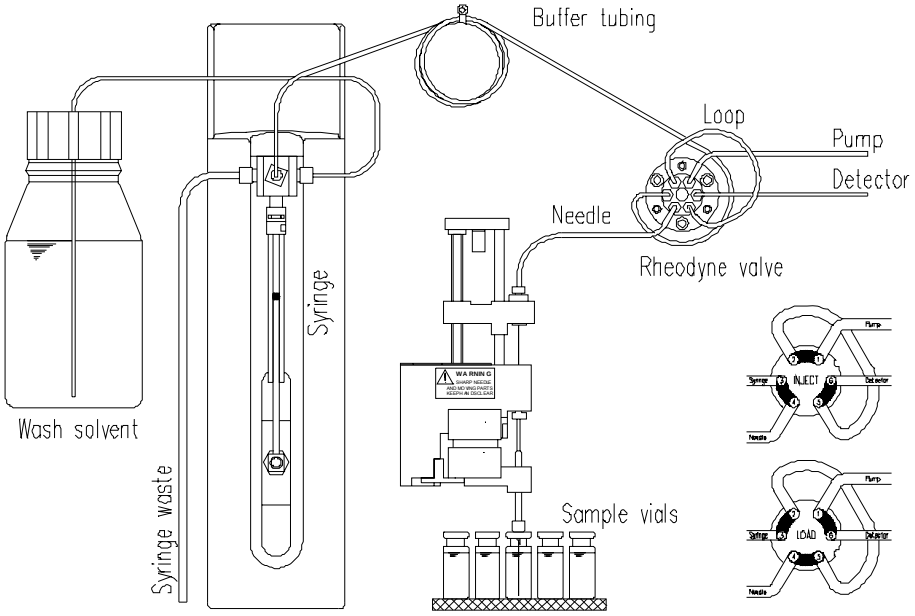
Trouble shooting



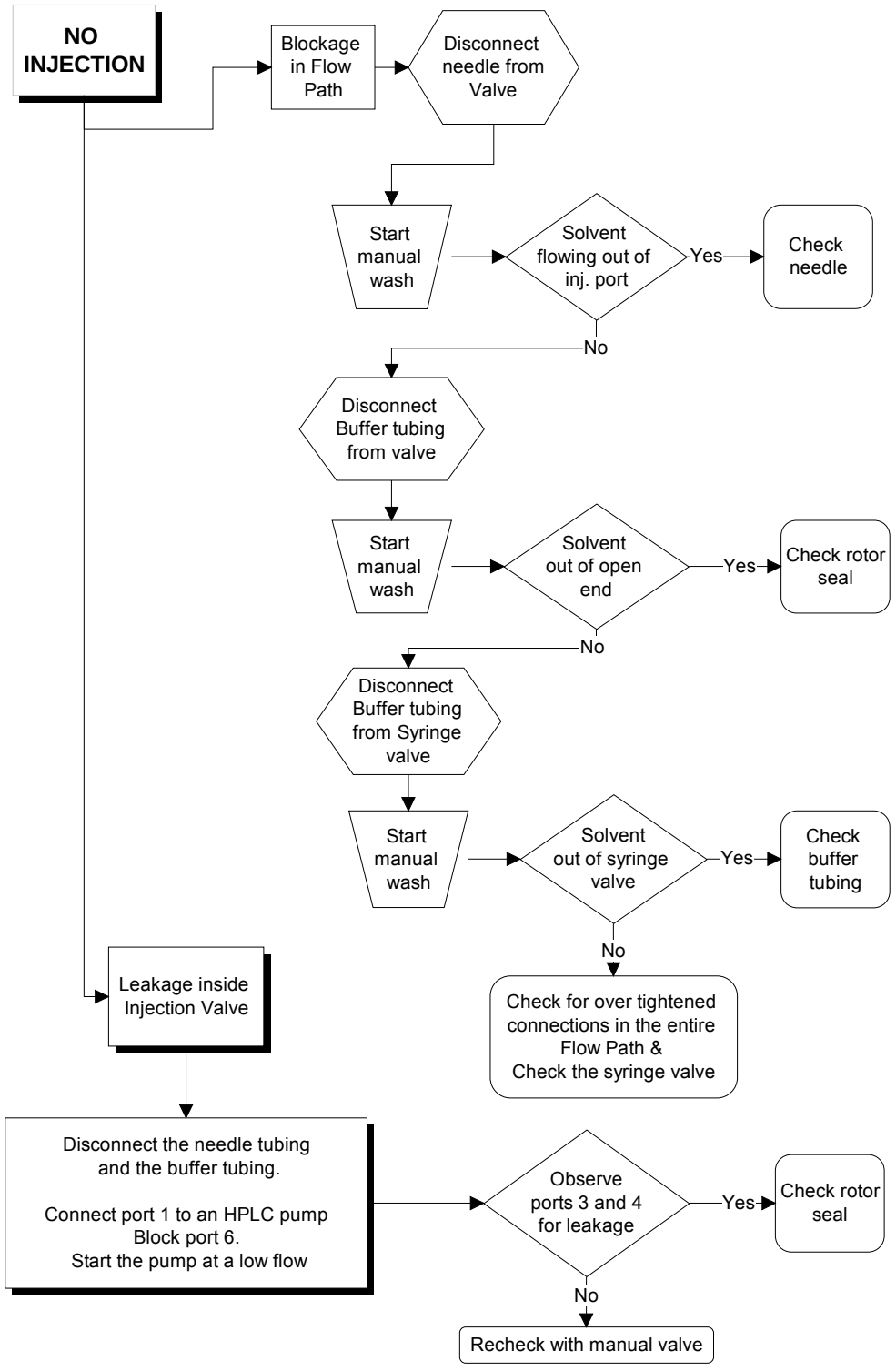
Analytical problems

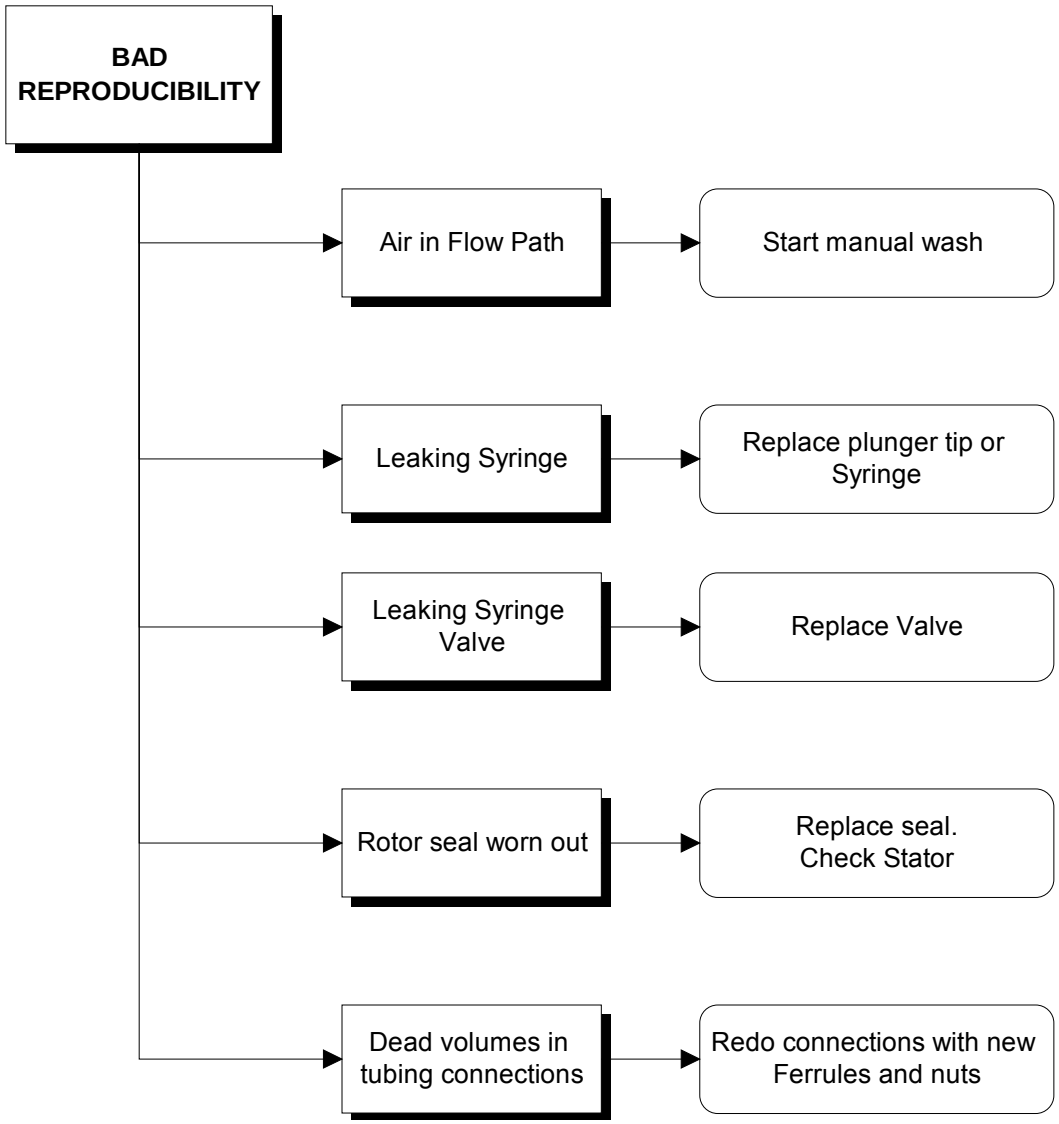
In case of analytical problems you will have to determine whether they are caused by the autosampler or by the rest of the system.

Quick check!
Replace the valve by a manual injection valve to discriminate between valve problems and other problems. Perform a number of manual Flushed loop injections. If the results are good, the autosampler is faulty; if not, the HPLC system should be checked.



In the flowcharts on the next pages it is assumed that the AS 100 does not display any error messages.
Please keep in mind that analytical problems might be caused by external influences, like temperature and/or light-sensitive samples. For this reason it is important to make sure that the application was running without problems before and that no changes have been made in the settings (System Menu).


NO INJECTION
Blockage in Flow Path
Disconnect needle from Valve
Start manual wash
Solvent flowing out of inj. port
Yes
Check needle
No
Disconnect Buffer tubing from valve
Start manual wash
Solvent out of open end
Yes
Check rotor seal
No
Disconnect Buffer tubing from Syringe valve
Start manual wash
Solvent out of syringe valve
Yes
Check buffer tubing
No
Check for over tightened connections in the entire Flow Path & Check the syringe valve
Leakage inside Injection Valve
Disconnect the needle tubing and the buffer tubing.
Connect port 1 to an HPLC pump
Block port 6.
Start the pump at a low flow
Observe ports 3 and 4 for leakage
Yes
Check rotor seal
No
Recheck with manual valve


BAD REPRODUCIBILITY
Air in Flow Path
Start manual wash
Leaking Syringe
Replace plunger tip or Syringe
Leaking Syringe Valve
Replace Valve
Rotor seal worn out
Replace seal. Check Stator
Dead volumes in tubing connections
Redo connections with new Ferrules and nuts

A P P E N D I X J

Logbooks

The following pages contain sample pages for logbooks that can be used with the AS 100. Keep a record of settings (System Menu) and of programmed methods and templates (Methods Menu) for future reference. Use copies of the provided pages.

User information

Name of user

Company

Department

Address

Telephone

Telefax

AS 100 information

Serial number

Firmware version

Purchase date

Installed options

Local dealer

Service engineer

Address

Telephone

Telefax

Comments:

System Menu settings

<GENERAL>			**<USAGE>**	
Loop volume		µL	Protection code:	.....
			Timebase methods	☐enabled
Needle tubing volume		µL		☐disabled
Syringe volume		µL	Mix methods	☐enabled
				☐disabled
Syringe speed	....	factor		
			User program	☐enabled
Needle height		mm		☐disabled
Skip missing vials	☐yes	☐no	User program protection	
			code	.....
Air segment	☐yes	☐no		
			Labelled vials	☐enabled
Headspace pressure	☐yes	☐no		☐disabled
Time display	HH:MM:SS	H:MM:mm	Templates	☐enabled
				☐disabled
Key click	☐on	☐off		
Error beep	☐on	☐off	Calibration vials	☐enabled
Alarm buzzer	☐on	☐off		☐disabled

<TRAY>	**<IO>**		
☐ 1: type /number	Inject-marker pulse length		....sec.
☐ 2: type /number			
☐ 3: type /number	Vial-marker pulse length		....sec.
☐ 4: type /number			
☐ 5: type /number	Labelled vial-marker pulse length		
☐ 6: type /number			....sec.
☐ 7: type /number	Input edge next injection		
☐ 8: type /number		☐falling	☐rising
	Input edge next vial		
position first transport vial:		☐falling	☐rising
position last transport vial:	Freeze input active		
		☐low	☐high
	Reset outputs after last series		
		☐yes	☐no
<CLOCK>	**<COMM.>**		
☐On (yy/mm/dd and hh/mm)	Device identifier: 2..		
☐Off			

Comments:

Templates

Template number	Injection method	Mix method	Wash method	Timebase method	User program Y/N	Comments
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						

Comments:

Injection methods

injection method number	type flushed partial pick-up	Anal. time	Flush vol.	Inj. per vial	Injection volumes:								
					1	2	3	4	5	6	7	8	9
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													
20													
21													
22													
23													
24													

Comments:

Wash methods

Wash method number	Wash between	Wash volume	Comments:
1	☐injections ☐vials ☐series		
2	☐injections ☐vials ☐series		
3	☐injections ☐vials ☐series		
4	☐injections ☐vials ☐series		
5	☐injections ☐vials ☐series		

Comments:

Timebase methods

Method number

	Action	Time		Action	Time
AUX 1	1 AUX-1 ON AT TIME:		ISS-A	1 ISS-A POSITION 6-1 AT TIME:	
	1 AUX-1 OFF AT TIME:			1 ISS-A POSITION 1-2 AT TIME:	
	2 AUX-1 ON AT TIME:			2 ISS-A POSITION 6-1 AT TIME:	
	2 AUX-1 OFF AT TIME:			2 ISS-A POSITION 1-2 AT TIME:	
	3 AUX-1 ON AT TIME:			3 ISS-A POSITION 6-1 AT TIME:	
	3 AUX-1 OFF AT TIME:			3 ISS-A POSITION 1-2 AT TIME:	
	4 AUX-1 ON AT TIME:			4 ISS-A POSITION 6-1 AT TIME:	
	4 AUX-1 OFF AT TIME:			4 ISS-A POSITION 1-2 AT TIME:	
AUX 2	1 AUX-2 ON AT TIME:		ISS-B	1 ISS-B POSITION 6-1 AT TIME:	
	1 AUX-2 OFF AT TIME:			1 ISS-B POSITION 1-2 AT TIME:	
	2 AUX-2 ON AT TIME:			2 ISS-B POSITION 6-1 AT TIME:	
	2 AUX-2 OFF AT TIME:			2 ISS-B POSITION 1-2 AT TIME:	
	3 AUX-2 ON AT TIME:			3 ISS-B POSITION 6-1 AT TIME:	
	3 AUX-2 OFF AT TIME:			3 ISS-B POSITION 1-2 AT TIME:	
	4 AUX-2 ON AT TIME:			4 ISS-B POSITION 6-1 AT TIME:	
	4 AUX-2 OFF AT TIME:			4 ISS-B POSITION 1-2 AT TIME:	
AUX 3	1 AUX-3 ON AT TIME:		SSV	1 SSV PORT: AT TIME:	
	1 AUX-3 OFF AT TIME:			2 SSV PORT: AT TIME:	
	2 AUX-3 ON AT TIME:			3 SSV PORT: AT TIME:	
	2 AUX-3 OFF AT TIME:			4 SSV PORT: AT TIME:	
	3 AUX 3 ON AT TIME:			5 SSV PORT: AT TIME:	
	3 AUX 3 OFF AT TIME:			6 SSV PORT: AT TIME:	
	4 AUX 3 ON AT TIME:			7 SSV PORT: AT TIME:	
	4 AUX 3 OFF AT TIME:			8 SSV PORT: AT TIME:	
AUX 4	1 AUX 4 ON AT TIME:		CODE	1 CODE-OUT: AT TIME:	
	1 AUX 4 OFF AT TIME:			2 CODE-OUT: AT TIME:	
	2 AUX 4 ON AT TIME:			3 CODE-OUT: AT TIME:	
	3 AUX 4 OFF AT TIME:			4 CODE-OUT: AT TIME:	
	3 AUX 4 ON AT TIME:			5 CODE-OUT: AT TIME:	
	3 AUX 4 OFF AT TIME:			6 CODE-OUT: AT TIME:	
	4 AUX 4 ON AT TIME:			7 CODE-OUT: AT TIME:	
	4 AUX-4 OFF AT TIME:			8 CODE-OUT: AT TIME:	
			END	END OF TIMED EVENTS AT:	

Mix method

Method number:											
Line	Action	Value	Position	Speed	Height	Line	Action	Value	Position	Speed	Height
1						41					
2						42					
3						43					
4						44					
5						45					
6						46					
7						47					
8						48					
9						49					
10						50					
11						51					
12						52					
13						53					
14						54					
15						55					
16						56					
17						57					
18						58					
19						59					
20						60					
21						61					
22						62					
23						63					
24						64					
25						65					
26						66					
27						67					
28						68					
29						69					
30						70					
31						71					
32						72					
33						73					
34						74					
35						75					
36						76					
37						77					
38						78					
39						79					
40						80					

User program

Method number:											
Line	Action	Value	Position	Speed	Height	Line	Action	Value	Position	Speed	Height
1						41					
2						42					
3						43					
4						44					
5						45					
6						46					
7						47					
8						48					
9						49					
10						50					
11						51					
12						52					
13						53					
14						54					
15						55					
16						56					
17						57					
18						58					
19						59					
20						60					
21						61					
22						62					
23						63					
24						64					
25						65					
26						66					
27						67					
28						68					
29						69					
30						70					
31						71					
32						72					
33						73					
34						74					
35						75					
36						76					
37						77					
38						78					
39						79					
40						80					

Do not place or remove a tray segment at the rear half of the AS 100 tray, as this may damage the tray sensors.

A P P E N D I X K

Getting started, using keyboard control

This chapter describes how to get started with the AS 100. The following will be explained:

- menus of the AS 100
- convenient working order
- methods that can be programmed and linked to series
- executing a series.

Menus of the AS 100

The software of the AS 100 is menu-driven. Important menus are:

- **Ready Menu**: this menu appears after the AS 100 has been switched on. It offers very general possibilities for among other things methods management and communication with other apparatus.
- **System Menu**: this menu appears after the **System** key in the keypad has been selected. It offers general possibilities for entering settings for an analytical run. Please note that settings may have been factory-installed to suit particular wishes of the user. In that case no changes will have to be made in the System Menu. You are advised to enable only those facilities that you will need, to ensure that menus are as concise as possible.
- **Methods Menu**: this menu appears after the **Methods** key in the keypad has been selected. The menu allows you to program a method to be used in an analytical run and to assign a number to it.
- **Series Menu**: this menu appears after the **Series** key in the keypad has been selected. The menu allows you to define a series, and to assign a number and a method to it for the analytical run.

Use the following **keys** to go through the menus:

E	ENTER; to confirm a choice or to select a choice made in a screen, or to step through menu lines
cursor keys	to change values in a field or to move to a different field in a screen
Escape	to return to a previous menu
CL	to remove a value from a field and enter NONE or DEFAULT
soft function keys	labels displayed in bottom line of the screen; to go to submenus

If [MENU] or [MN] is displayed in the top right hand corner of the screen, you can press the **Menu** key in the keypad to display more possibilities offered by the menu. Refer to chapter 1 for an explanation of all keys in the keypad.

Convenient working order

After you have determined what type of analytical run you want to perform, the most convenient working order for the AS 100 is:

6. Enter settings in the System Menu. Note that the settings for the AS 100 probably already have been correctly entered (factory-installed).
7. Program a method for the analyses you wish to perform in the Methods Menu.
8. Define a series and link a programmed method to a range of vials in the Series Menu.
9. Execute the series.

Please note that it is allowed to use a different order; however, you should keep in mind that settings entered in the System Menu determine which possibilities appear in the Methods and Series menus. Refer to chapter 5 for more information on specific items in menus and the way they influence the other menus.

Types of Methods and links to Series

The AS 100 offers the following types of methods for different parts of the sample handling routine:

- **injection** method: contains information on the injection routine, flush volume and analysis time.
- **wash** method: describes a wash volume and when a wash must be executed.
- **mix** method: a pre-injection method in which additional sample handling can be performed (e.g. pre-column derivatisation).
- **time base** method: a post-injection method with which outputs to other devices (e.g. integrator or pump) and switching of the ISS valve are controlled.
- **user program**: offers the possibility to program sequences of all actions that can be executed by the AS 100 in separate steps.

Each programmed method is assigned a number. The AS 100 offers the possibility to store a combination of defined methods in a **template**. A template is also identified by a number.

Methods must be linked to series before they can be used. The following possibilities are offered by the AS 100:

- You can assign an individual **method** to a series: methods (mix, injection, wash, timebase) can be linked to vials in a series.
- You can assign a **template** to a series: a combination of various programmed methods (mix, injection, wash, timebase) can be defined in a template. The template is linked to a range of vials in a series. In this way all steps in an analytical run are laid down and stored.
- You can assign a **user program** to a series: a possibility to combine all possible steps in the analytical process in one program. The user determines the order of the separate actions the AS 100 has to perform.

Executing a Series

Execution of a series is only possible if you have programmed a method and defined a series for the samples you wish to analyse. Series are not stored in battery backup and exist only for as long as the AS 100 is switched on.
Execute the following steps:

4. Start programming the series by pressing **Start/Stop**.
5. Enter the number of the first series to perform and the number of the last series to perform.
6. Select <START> to start the actual analytical run. The AS 100 starts to execute the series you have defined.

After the AS 100 has executed the run, the Ready Menu will appear again. Refer to Chapter 4 for a number of examples illustrating this working order.

It is possible to program series and methods during a run. Press **Series** or **Methods**; the possibilities offered in the menus are identical to those offered when the AS 100 is idle.

If a series or method is changed, the new values become active the next time the AS 100 starts a series. The series currently running are not affected by the changes.

Executing a Series in remote control

7. To execute a series from remote control, execute the following steps:

1. Press **Start/Stop**.
2. Enter the number of the first and the last series to be performed.
3. Select <REMOTE> to enter the remote control mode. The AS 100 will now operate as slave of another device and can be controlled with Next injection input and Next vial input. To indicate that remote control is active, an “r” is displayed in the bottom left corner of the display

during execution of the series. At the end of the series the message “Series completed via remote control” is displayed.

4. Press **Escape** to return to the Ready Menu.

A P P E N D I X L

Using the AS 100 with keyboard control

This chapter describes a number of examples of actions that can be performed with the AS 100. Please note that this chapter does not describe all types of actions that can be performed. Try to do these examples to learn to work with the AS 100.
These examples can be executed after the AS 100 has been installed in accordance with Appendix A and after all items described in Chapter 2 have been correctly set up.

Example 1: 10 µL partial loop fill injection

After the AS 100 has been switched on, an initialisation procedure is executed. In this example it is assumed that a loop of 100 µL, needle tubing of 15 µL, a syringe of 250 µL and a type A tray has been installed. Wait until the Ready Menu appears on the screen, and then execute the following steps:

For system settings:

Press keys	Description
System	to enter the System Menu
<GENERAL> **E**	to enter the General Menu
[0100] **E**	to define the volume of the installed loop
[015] **E**	to define the volume of the needle tubing
<250> **E**	to define the volume of the syringe
<NORMAL> **E**	to set syringe speedto set sample needle height to 2 mm
[02] **E**	to enable use of air segment
<YES> **E**	to switch headspace pressure on
<YES> **E**	to return to the Ready Menu
Escape Escape	

For this example all other settings used will be default.

To program a method:

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [01] **E**	program injection method number 1
<PARTIAL> **E**	to select partial loop fill injection method
[100] **E**	to define an analysis time of 1 minute
[030] **E**	to define a flush volume of 30 µL
[1] **E**	to define the number of injections per vial
[10] **E**	to set the injection volume at 10 µL
Escape Escape	to return to the Ready Menu

To define the series:

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to define the injection method number
CL E	to enter <NONE> for wash method
[01] **E**	to define location of the first sample vial
[01] **E**	to define location of the last sample vial
Escape	to return to the Ready Menu

To run the series:

Place a sample in position A 1 of the tray.

Press keys	Description
Start/Stop	to start the AS 100
[01] **E**	to start at series number 1
[01] **E**	to stop after execution of series number 1
<START>	to start the analytical run

The AS 100 will now locate vial A 01 and perform a 10 µL partial loop fill injection. The display of the AS 100 will indicate the status (Checking tray, Flushing, Loop fill, Running, Rinse buffer, Running). The display also indicates the number of the defined series (01), the method number (01) and the vial on which the analysis is performed (A 01).
At the end of the defined analysis time the Ready Menu will be displayed again to indicate that the AS 100 is ready for the next analytical run.

Example 2: 3 x 10 µL injection, µL pick-up, wash step

In this example a different injection method is used than in the previous one. For that reason several settings must be adapted in the System Menu.

For system settings:

Press keys	Description
System	to enter the System Menu
<GENERAL> **E**	to enter the General Menu
E until Air segment appears	to go to the Air segment field
<NO> **E**	to switch off air segment
Escape	to return to the System Menu
<TRAY> **E**	to enter the Tray Menu
E until transport vial appears	to go to transport vials field
<TYPE_A>	to select type A tray segment
[1] **E**	to define position of the first transport vial
[1]	to define position of the last transport vial
Escape Escape	to return to the Ready Menu

To program a method:

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [02] **E**	to define method number 02
<PICK-UP> **E**	to select the injection mode for this method
[100] **E**	to define the analysis time
[3] **E**	to define the number of injections per vial
[10] **E**	to define volume of 10 µL for 1st injection
[10] **E**	to define volume of 10 µL for 2nd injection
[10] **E**	to define volume of 10 µL for 3rd injection
Escape	to return to the Methods Menu
<WASH>	to enter the Wash Menu
[01] **E**	to define wash method number 01
<INJECTION>**E**	to select wash between injections
[300]	to define the wash volume
Escape Escape	to return to the Ready Menu

To define the series:

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the series number
[02] **E**	to define the injection method for this series
[01] **E**	to define the wash method for this series
[02] **E**	to define the location of the first sample vial
[02]	to define the location of the last sample vial
Escape	to return to Ready Menu

To run the series:

Put a vial with transport solvent (mobile phase) in transport vial position A 1 and a vial filled with sample at position A 2. Make sure the transport vial is correctly filled before starting a new series.

Press keys	Description
Start/Stop	to start the AS 100
[01] **E**	to start at series 01
[01] **E**	to stop after series 01
<START>	to start execution of the series.

At the end of the defined analysis time the Ready Menu will appear again to indicate that the AS 100 is ready for the following next run.

Example 3: 10x dilution, 10 µL partial loop fill injection

This example describes how to let the AS 100 transfer 360 µL from Reagent A to the destination vial, add 40 µL of sample, mix 3 times with 250 µL and subsequently inject 10 µL.

For system settings:

Press keys	Description
System	to enter the System Menu
<USAGE> **E**	to enter the Usage Menu
E E	to go to the Mix field
<ENABLED>	to enable use of mix methods
Escape Escape	to return to the Ready Menu

As soon as a change has been entered in the System settings, the message “ALL SERIES DEFAULT” appears. The user will have to redefine series because the settings have been changed.

To program the injection method:

Press keys	Description
Methods	to enter the Methods Menu
<INJECTION> [03] **E**	to enter the Injection Menu
<PARTIAL> **E**	to select partial loop fill injection mode
[100] **E**	to define the analysis time
[50] **E**	to define the flush volume
[3] **E**	to define the number of injections per vial
[10] **E**	to enter the injection volume for 1st injection
[10] **E**	to enter the injection volume for 2nd injection
[10] **E**	to enter the injection volume for 3rd injection
Escape	to return to the Methods Menu

To program the mix method:

Press keys	Description
<MIX>	to enter the Mix Menu
[1] **E**	to define Mix method number 1
<INSERT>	to define mix method step number 1
<ASPIRATE> [5] <AIR> **E**	to aspirate an air segment of 5 µL
<INSERT	to define mix method step number 2
<ASPIRATE> [225]	to aspirate 225 µL
Menu <REAG-A> **E**	to perform defined asp. From reagent vial A
<INSERT>	to define mix method step number 3
<DISPENSE> [180] ▸ ▸ [02] **E**	to dispense 180 µL to destination vial
<INSERT>	to define mix method step number 4
<DISPENSE> [50] <WASTE> ▸ [5] **E**	to dispense 50 µL
<INSERT>	to dispense defined volume to waste
<REPEAT> [1] ◂ [4] **E**	to define mix method step number 5
<INSERT>	to repeat last four steps once
<ASPIRATE> [5] <AIR> **E**	to define mix method step number 6
<INSERT>	to aspirate an air segment of 5 µL
<ASPIRATE> [60] <SAMPLE> **E**	to define mix method step number 7
<INSERT>	to aspirate 60 µL of sample
<DISPENSE> [40] <DESTINATION> ▸ ▸ [2] **E**	to define mix method step number 8 to dispense 40 µL to destination vial
<INSERT>	to define mix method step number 9
<DISPENSE> [50] <WASTE> ▸ [5] **E**	to dispense 50 µL to waste
<INSERT>	to define mix method step number 10
<ASPIRATE> [50] <AIR> **E**	to aspirate an air segment of 50 µL
<INSERT>	to define mix method step number 11
<ASPIRATE> [200] <DESTINATION> ▸ [3] **E**	to aspirate 200 µL from the destination vial
<INSERT>	to define mix method step number 12
<DISPENSE> [200] ▸ [9] **E**	to dispense 200 µL to the destination vial
<INSERT>	to define mix method step number 13
<REPEAT [3]	to repeat the last 2 steps three times
Escape Escape	to return to the Ready Menu

To define the series:

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define series number 1
[01] **E**	to select Mix method number 1 for this series
[03] **E**	to select Injection method number 3
CL E	to select <NONE> for wash method
[1] **E**	to define location of first sample vial
[1] **E**	to define location of last sample vial
[2] **E**	to define location of first destination vial
[3] **E**	to define position of Reagent A
Escape	to return to the Ready Menu

To run the series:

Put sample in position A 01; position A 02 is used as empty destination vial. Place a filled reagent vial in position A 03. Make sure the reagent vial is filled correctly before starting a new series.

Press keys	Description
Start/Stop	To start the AS 100
[01] **E**	To start at series number 1
[01] **E**	To stop after series number 1
<START>	To start processing of sample

The AS 100 will now start searching for the Reagent vial and transport 180 µL to the destination vial twice, then 40 µL of sample will be added and after mixing 3 times a 10 µL injection will be performed.

Example 4: defining a template, adding protection code

This example describes how to incorporate the injection method (02) and wash method (01) defined in example 2 in a template. A protection code will be added.

For system settings:

Press keys	Description
System	To enter the System Menu
<USAGE> **E**	To enter the Usage Menu
[123456] **E**	To enter a 6-digit code (memorize code!)
E	to go to the mix methods field
<DISABLED> **E**	to disable use of mix methods
E E	to go to the template field
<ENABLED>	to enable templates
Escape Escape	to return to the Ready Menu

After use of templates has been enabled the message "ALL SERIES DEFAULT" appears. The user will have to redefine series because the settings have been changed.

To select the methods to be incorporated in the template:

Press keys	Description
Methods	to enter the Methods Menu
[123456] **E**	to enter the methods protection code
<TEMPLATE>	to enter the Template Menu
[01] **E**	to define the number for the template
[02] **E**	to define the injection method for this template
[01]	to define the wash method for this template
Escape Escape	to return to the Ready Menu

To define the series:

Press keys	Description
Series	to enter the Series Menu
[01] **E**	to define the Series number
[01] **E**	to define the Template method number
[01] **E**	to define the first sample vial
[02] **E**	to define the last sample vial
Escape	to return to the Ready Menu

To run the series:

Press keys	Description
Start/Stop	to start the AS 100
[01] **E**	to start analysis at series 01
[01] **E**	to stop after analysis of series 01
<START>	to start the analytical run

The AS 100 now performs the same actions as in Example 2, except that analysis is performed on two vials: A 01 and B 01.
Note: select <DEFAULT ALL> in the Ready Menu (Utilities Menu) to erase all series and methods defined in these examples and to default all settings.

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