3000 EVOLUTION

Auto Analyzer for Biochemical Tests

USER'S GUIDE

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RELEASE HISTORY

RELEASE	DATE	UPDATES and MODIFICATIONS
_3	4/11/2005	Inserted Explanation about Extended multistandard
_4	29/12/2005	Inserted Appendix about WEEE and RoHS Directives Corrected explanation about ID changing, test parameter printing Inserted explanation about default temperature parameter
_5	09/01/2006	Changed result visualization on Body Window for End point and Kinetic test
_6	06/09/2006	Added Appendix about Biohazard Risk
_7	26/11/2007	Added Appendix about printing layout
_8	23/11/2012	Updated default airgap value
_9	9/03/2016	Clarification on blank save mode and initialization, improved troubleshooting, reduced reading height to 1000 uL.
_10	01/07/2016	Modified logo
_11	12/03/2019	Modified logo
_12	13/07/2020	Added acoustic signal in air aspiration

INTRODUCTION

The *3000 Evolution* is an interferential filter analyzer, completely managed by microprocessors. This instrument has been designed to perform spectroscopic measurements at predetermined wavelengths of analyte concentration and enzyme activity using various reagents. It performs optical measurements and processes them according to programs with parameters that can be entered by the operator. It executes, in a rapid and precise manner, most of important chemistry and hematology tests. Particularly, the following determinations can be carried out:

ABSORBANCE

END-POINT

KINETICS

FIXED-TIME

MULTISTANDARD

DIFFERENTIAL

The instrument is equipped with a two-way flow cell system; it ensures low carry-over values even with limited sample volumes. Disposable macro and micro cuvettes (glass or plastic), with an optical path of 1 cm, can be used by simply removing the flow cell from the reading compartment and placing it on the right-side one. Seven filters (and one additional empty position) are included in the instrument. The selection of the interferential filter is automatic, with powered handling managed by a microprocessor. This feature makes reading easier and eliminates filter selection errors that may occur in instruments which have a manual selection.
The optical part is very sophisticated: it consists of a high-power halogen lamp (20 W) whose light beam is centered by a quartz lens, thus allowing a high accuracy in measurements even when reduced-volume cuvettes are used.
A 10-position dry incubator, which can contain both square and cylindrical cuvettes, allows the sample incubation before reading. The temperature of the incubator is equal to the one of the reading cell and it is selectable from 20°C to 40°C.
The execution of the analyses and instrument programming are simple and performed by means of a keyboard, following the instructions shown on the display. This display also shows the status and error/fault messages. The analytical results are directly displayed in the measuring units selected by the current program.
The language of instructions can be selected between English and other customizable languages.
The instrument is provided with a 24-columns thermal printer that can print analytical results as well as programparameters (multistandard and kinetics results are also available in a graphic plot). All printed information is sent to a serialRS-232 standard output. The printer can be also completely disconnected as well as can be disabled the graphic plot.
An advanced software guides and controls all operations carried out by the user. An acoustic signal further helps the user, by emitting a sound of a different tone from the usual one in case you pushed a wrong key.
A hundred and twenty programs can be stored. To carry out an analysis, it is important to enter all the parameters correctly, including the K and standard values where necessary.
The instrument is supplied already programmed. Anyway, it is advisable to check that the entered parameter values correspond to those stated in the methods.
A modification of the analytical parameters can be done by the operator before carrying out the analysis.

The instrument is entirely re-programmable: however, the HCT and ERY programs (IF PRESENT), are reserved for hematocrit and erythrocytes determinations; thus they must not be used for other methods (these programs have a specific software for their corresponding Dyaset products).

The instrument has an internal memory that can store up to 400 result. Tests are automatically stored in memory every time they are executed. Results can be recalled in every moment in the right section of the program manager. In this way the instrument can printout result as a batch or a profile analyzer.
The instrument is provided with two level QC program, available for 30 independent tests.

1 HOW TO USE THE MANUAL

In order to understand this manual better, the instrument functions are explained in a schematic way, referring to examples. These examples refer to display's messages and explain which button have to be pressed to change settings or execute procedures.

The following picture show and describe the display's layout:

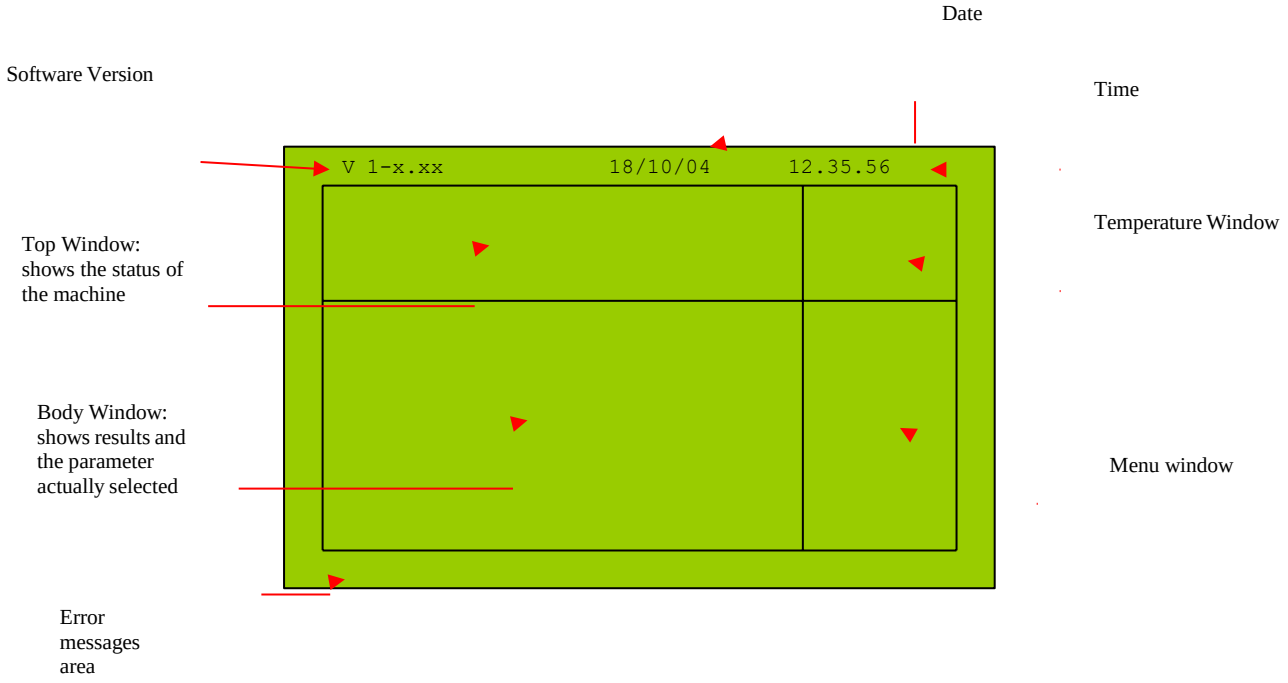


Fig.1 Display's Layout

This symbol is for Warning or Caution Advises: they are related to user and/or instrument's safety

2 INSTRUMENT INSTALLATION

2.1) Unpacking the instrument

Check if the package is in perfect condition and with the original seals intact. If the package shows any serious damage, it may have suffered from improper handling: contact your dealer for instructions.

The box should contain, besides this manual, the following items:

1) the 3000 Evolution instrument;
2) the instrument dusty-cover;
3) the power cable;
4) a plastic waste bottle;
5) 2 rolls of printing paper;
6) one meter of plastic pipe;
7) a spare 20W/12V halogen lamp already cabled;
8) 2 spare 2A fast fuses;
9) a box with 100 1cm optical-path cuvettes;
10) the Release Protocol;
11) the Warranty document;

Do not lose the original envelope and package since they are required in case of moving or shipping the instrument.

2.2) Instrument description

Figure 2 shows the main parts of the instrument:

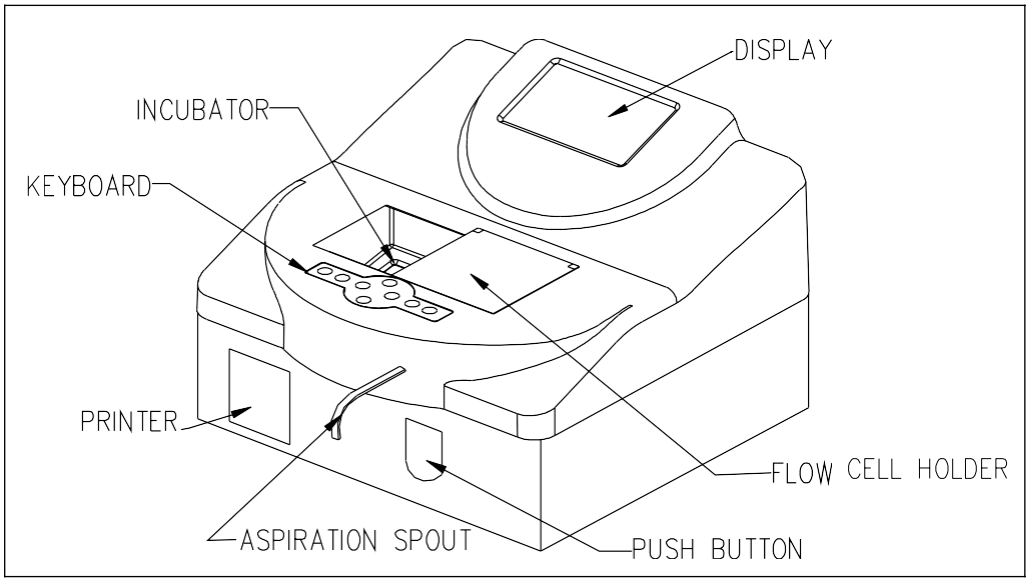


Figure 2: Global view of the instrument

Front side:

Display. It is graphic 240x128 pixel back-illuminated liquid crystal type, consisting of two 16-character lines. Usually, the first line displays the main parameters of the analysis in progress (analysis number, item and K). The second line guides the

operator in performing the analysis in an interactive way. Furthermore, the instrumental status and error/fault messages are also displayed.

Printer. It is a 24-columns thermal type. It can be enabled or disconnected by the operator and can print analytical results and parameters. In multistandard and kinetic analysis it can also plot a graphic of the results.

Incubator. It consists of a single thermostated aluminum block with 10 housings for cylindrical and prismatic cuvettes, arranged on three rows of three, plus another position on the right side of the reading cell. Thermostating is obtained by means of semiconductor devices which ensure an accurate regulation of the temperature. The two housings, separated from the previous ones, are: a reading cell compartment (on the left) and the another thermostated compartment where to place the flow cell when another type of cuvette is used for reading.

Aspiration spout. A Teflon tube which protrudes from the front panel of the instrument and intakes the sample into the flow cell.

Sample lever (Push Button). Situated under the aspiration spout. When pressed, it turns on the peristaltic pump to intake the sample.

Flow cell. The instrument can read either in flow cell or in standard cuvette. Instrument is equipped with 18 µL flow cell. Pay attention to the polarity of the flow cell when you insert it in the reading compartment. Be sure that the face with the white arrow is towards the operator.
The inclination of the flow cell inside the reading compartment is optimal since it ensures that no air bubbles are formed inside the cell itself. To clean the cell **inside**, press WASH key aspirating sodium hypochlorite and air. A diluted solution of sodium hypochlorite or a liquid detergent for glassware is recommended to clean the flow cell **outside**.

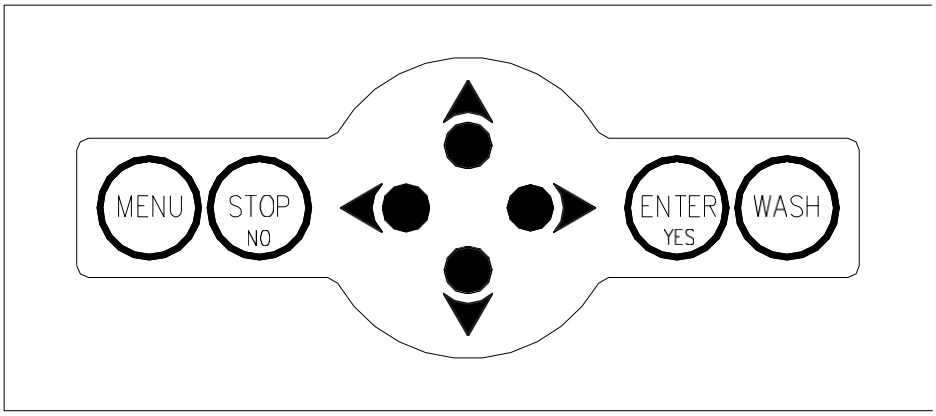


Figure 3: Close view of the keyboard

Keyboard. The keyboard (Figure 3) is used in a functional way, for moving inside the Menu. Data entering is guided by the emission of a beep when a key is pressed. The keyboard consists of 8 keys, 4 of which are arrow, for moving inside of the Menu and 4 functional. Two keys (ENTER/YES and STOP/NO) are both functional for two kind of operation. The following table shows which functions can be entered by pressing each of the functional keys:

FUNCTION KEYS	ACTION
MENU:	To enter inside the MENU

ENTER/YES:	Store the entered values. Confirm.
STOP/NO:	Interruption of the operations in progress. (always active key).
WASH:	Washing the flow cell. Connects the peristaltic pump continuously. This key functions only when the instrument is on the main menu.
	Right movement arrow
	Left movement arrow
	Down movement arrow
	Up movement arrow

You can also connect a PS-2 keyboard to the instrument through the proper connector and interact with the software.

Instrument back side:

In the back side of the instrument (Figure 4) you can find the following items:

- RS-232 D-type 9-pin male connector for connection with an external serial printer or PC through the serial port. Refer to the Appendix C of this manual for the serial transmission protocol used by the instrument to output results. You can use the specific software to connect the instrument to a PC: ask your dealer for further details.
- PS2 connector to connect a keyboard to 3000 Evolution
- Serial number of the instrument and K-factor for HCT and ERY tests. This two tests are special and can be used only with the K-factors specified in this label.
- ON/OFF switch to turn ON and OFF the instrument.
- Cooling fan.
- Fuse holder, containing number 2 fuses. Refer to technical specification paragraph for fuses values.
- Waste connector: attach here the waste tank pipe.

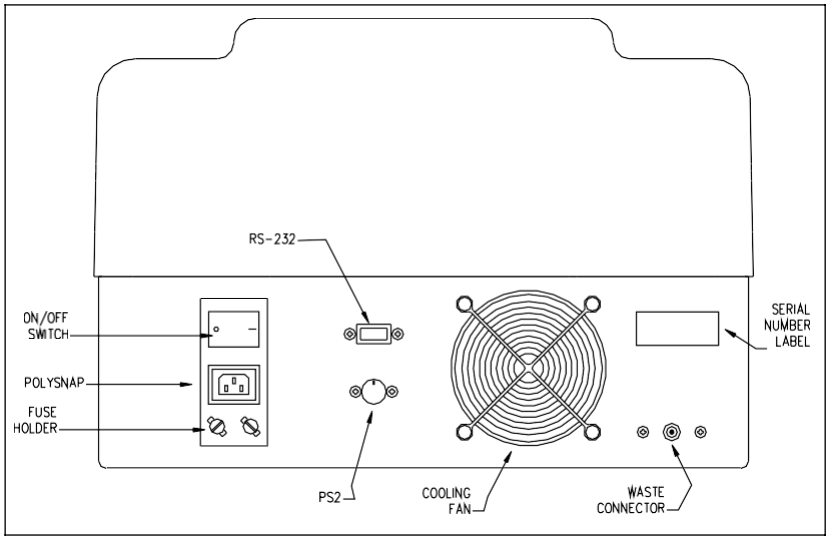


Figure 4: View of the back side of the instrument

2.3) First installation of the instrument

This procedure allows the User to install the instrument. Please, in the case of any doubt or ambiguity in understanding this procedure, contact our nearest distributor since an improper installation may damage seriously the instrument.
The instrument can work either with 220 V – 50 Hz (working range is from 170 to 264 V) power supply or 110 V – 60 Hz (working range is from 85 to 132 V) and automatically identifies the applied power supply.

WARNING: make sure the chosen supply socket has a suitable earth connection, since it is required to assure user's safety during instrument usage.

. Place the instrument on a stable and vibration-free support. Avoid its placing near heat sources (e.g. heaters, ovens, under high power lamps), under direct sunlight, near strong electromagnetic sources (e.g. motors) or with the instrument's back close to a wall, which would block the cooling air flow. The operational temperature range is 15-30Cº and humidity must be under 80%.

- Before connecting the instrument to the power supply, make sure that it is switched off. In this case, the switch on the polysnap module in the backside of the instrument must be in the 0 position.
- Connect the waste discharge tube to the outlet on the instrument back panel and place its cap inside the waste tank.
- Remove the stick on the flow cell compartment and open it.
- Open the flow cell compartment and remove the protection used for the package of the flow cell.
- Switch on the instrument: the display will show the number of software version, the date and the time in their correct position (see Fig. 1).
- Then, as requested by the message on the display, put the aspiration spout inside about 1.5 ml of distilled water (this quantity can be not exact, it is only used for internal blanking and to initialize the flow cell) and press the 'Push' button on the front side of the instrument: the instrument enters the Self -test.

- Wait for Self-test to complete: the instrument performs an auto-diagnostic test which should end, if no error occurs, prompting for date and time setting. If Self-test is not successful, the error message is hard-copied on the printer: in this case, refer to Appendix A (Troubleshooting) for further details and possible solutions.

- Set date and time (refer to the paragraph 2.1)

- When the main prompt is displayed start aspirating distilled water by pressing the WASH key until the whole hydraulic circuit is full with water (i.e. distilled water begins to flow out of the waste connector going in to the Waste Tank).

- If you have any problem during this step (like pump seems not to have enough power to aspirate this distilled water) you can use a syringe (Figure 5) to inject the first 5 ml water directly in the aspiration spout, while pressing the WASH key to keep the peristaltic pump motor running. In such a way you will initialize the peristaltic pump hydraulic and the flow cell, and you will be able to aspirate normally using the WASH button and the SAMPLE LEVER (PUSH BUTTON).

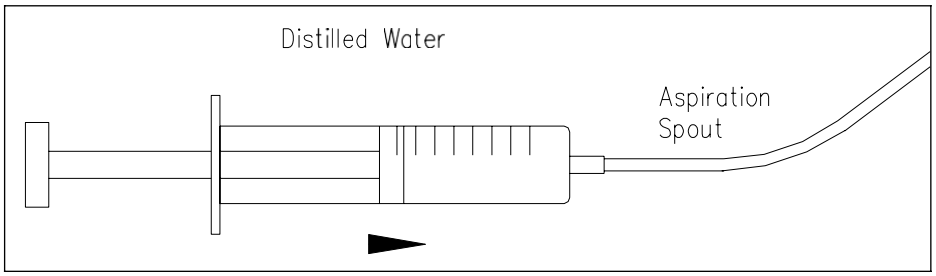


Figure 5: External injection of water towards the peristaltic pump

- Aspirate sodium hypochlorite (concentration between 6% and 10%) using the WASH key, for a volume amount of 20-30 ml.

- Aspirate distilled water using the WASH key for a volume amount of 20-30 ml.

- Now the instrument is installed and ready to work.

IMPORTANT: WAIT AT LEAST 15 MINUTES BEFORE EXECUTING ANY ANALYSIS, THUS ALLOWING THE INCUBATOR TO REACH AN OPTIMAL THERMAL STATUS.

2.4) Instrument general maintenance

It's recommended by Dyaset srl to follow these operations for a correct maintenance of theinstrument:

- Avoid cleaning the instrument with water or alcohol. Use a dry cloth.
- A periodic cleaning of the reading cell is however advised, by means of aspiration of sodium hypochlorite. Wash flow cell by WASH key to clean internally flow cell and tubes. Use 10 mL of sodium hypochlorite (6 to 9% diluted) and WASH button, alternating air and washing solution.
- At the end of a working session is recommended to aspirate distilled water inside the flow cell in order to clean peristaltic pump and remove dirty and solution sedimentation outside flow cell. Use the WASH button and 10-20 ml of distilled water for this purpose. Leave the instrument with distilled water inside the flow cell during the night.

- If you plan not to work with the instrument for more than 3 days, prepare it for a long inactivity time (refer to the session below).
- Avoid dropping moisture and water into the reading hole and incubator.
- Clean the incubator and the reading hole with sodium hypochlorite or standard detergent (for example glass detergent).
- Clean the cover with detergent.
- Avoid dust and cover the instrument with its plastic cover when not in use.
- Avoid inserting objects into the cooling openings.

INACTIVITY PERIOD:

If the instrument has to be prepared for long inactivity time (more than one week), is recommended to follow this procedure:

- Aspirate sodium hypochlorite using the WASH key, for a volume amount of 20-30 ml.
- Aspirate distilled water using the WASH key for a volume amount of 20-30 ml.
- Completely deplete the instrument inside (this means press the WASH button and let the instrument aspirate air, until water no more flows out of the waste connector).
- Always use Dyaset Srl original package to pack/ship the instrument.
- Store the instrument between 0°C to 50 °C, avoiding moisture and wet place.

CAUTION: If the instrument is used differently and not as described above by the producer, its integrity could be compromised.

2.5) Daily maintenance (initialization procedure): preliminary operations before daily usage

At every power on, the instrument performs a calibration and a self-test, that is called initialization procedure. Because the wall of an empty flow cell may be dirty , which can result in bad transmission of the light beam, and to avoid the users to remove every time the flow cell, the instrument, as it is switched on, asks the user to insert 1.5 ml of distilled water in the aspiration spout to perform calibration and self-test. In the Body Window will appear the request:

```
Insert 1.5 ml
of distilled water
```

If you press SAMPLE LEVEL (PUSH BUTTON), the instrument automatically aspirates distilled water into the flow cell. This is recommended mode if you work with flow cell.
ONLY IF YOU WORK WITH DISPOSABLE CUVETTE: You press STOP, and instrument skips aspiration step. In this case reading hole must be empty, in order to blank against air..
Whatever is your choice the instrument automatically selects the 340 nm filter, displaying “BLANKING ” on the display. Then it performs its first reading, in order to get the value of the blank and complete its calibration. This operation is critical when blank saving feature is enabled, because the value of blank read here are used for calculating the reagentblank values recalled by the system memory.

Working mode	Reference blank	Operator action
Flow cell	Against water blank performed with flow cell in reading hole	Insert 1.5 mL (approx.) of distilled water and press PUSH button to aspirate.
Disposable cuvettes	Against air (reading hole empty)	Remove any cuvette from reading hole and press STOP

Thermostat is not connected in this status.

NOTE: After this calibration, wait 15 minutes to start operating with analyser, in order to stabilize lamp and temperature.

3 INSTRUMENT SETUP

NOTE: when you are using an external PS-2 keyboard, the "TAB" key corresponds to button MENU, the key "ENTER" corresponds to button ENTER/YES and the arrows' keys correspond to arrows' buttons.

3.1) Modifying settings

On switching on the instrument, after the self-test the display will show the Main Menu. From Main Menu you can reach Settings Menu to customize the instrument.

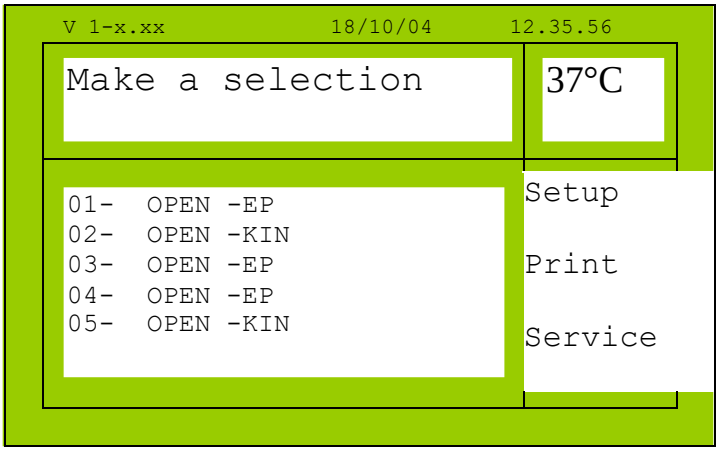


Fig. 6 Main Menu

Press Menu to shift the cursor in the Menu Window. The cursor now is on "SETUP": press ENTER/YES to select "SETUP". The Body Window will appear as follows:

```
Setting

Erasing
```

Press ENTER to enter in the Setting Menu and then scroll it it pressing UP or DOWN. Select the feature you want to change pressing ENTER button. The modifications will be done through the arrows' buttons. This is the complete list of the feature you can modify:

Set Language	Select the language of the instructions on the display
Set Date & Time	Set current date and time
Contrast	Select the contrast value for the display
Enable Print	Connect and disconnect the printer. When the printer is disconnected, it is not possible to execute any printing operation.
Enable Plot	Enable or disable the plot on kinetic and multistandard analysis. When the plot is disabled only analytical results and program parameters are printed (if printer is enabled).

Lamp Save 1	If Lamp Save is enabled, the lamp is automatically switched off when in main menu, the instrument is not used for 2 minutes
Print Method	Select OFF to disconnect the printer completely when entering into a a program; PRZ to obtain an automatic print of date, time, name, and analysis number, reference values and linear limits of the test, each time an analysis is selected, TOT to obtain an automatic printing of all stored parameters, each time an analysis is selected, REP to obtain an automatic printing of the test name every time a result is calculated.
Print Result	Enable or disconnect the automatic printing of analytical result
Number Rows	You can insert the number of white rows between one printed row and the next one.
Autoreset ID	If enabled ID number will be reset every time user executes a new test
Air aspiration	Select the air-gap volume. It is advisable to enter up to 00110 Above this volume air may enter into the cuvette.
Delay (s)	Enter the delay time between sample intake and air-gap intake.
Temperature (°C)	Enter the default Temperature. If it is different from 0°C the instrument reaches this temperature when is turned on and every time no test is selected

When a setting is modified, press ENTER to confirm. Then you can continue scrolling the Menu using UP and DOWN arrows and select a new feature which should to be changed. When you finish to modify the features, press STOP to exit from Setting Menu. On the Body Window appear the following message:

```
Save Changes?

YES=Enter NO=Stop
```

You have to press ENTER to save in memory the modifications or STOP if you don't want to save the changes.

3.2) Erasing data memory

If you want to erase old data stored in the memory, you have to enter the "SETUP" menu as explained in paragraph 2.1 and then select "ERASING". You enter in the Erasing Menu and the Body Window will have this feature:

```
Result mem erase

Memory not empty
```

Press ENTER to erase the memory, then STOP to exit and ENTER to save. If you select again "ERASING", the display will show the message "Memory Empty".Memory space is 400 test.

If you want to erase all QC data, when on the display appears the previous message, press DOWN arrow to scroll Erasing Menu: the message on the Body Window will be:

```
Initiate QC
```

Press ENTER if you want to erase memory or STOP to exit. If you press ENTER, a message will appear in the middle of the display, asking you if you want to erase memory. Press ENTER to confirm or STOP to preserve memory.

NOTE: When memory is full, test are overwritten starting from the older without prompting.

3.3) Connection/disconnection of the thermostat

The analyser doesn't enable automatically the thermostat, but the thermostat is automatically connected when the instrument is in test mode. The target temperature is that configured for the test. If you want to disconnect the thermostat, you have to select 00 when you select the temperature for the test (see chapter 8 for further details).

4 INSTRUMENT SERVICE

To enter the Service Menu, press MENU when you are in Main Menu, then select "SERVICE" using DOWN arrow and press ENTER. The Body Window appears as follows:

```
ABS Mode

Pump Calibration

Diagnostic & Service
```

4.1) ABS Mode

To select ABS Mode, enter Service Menu as explained above.
The analyser will ask to you, in sequence, to select the wavelength of the filter, the volume of the sample to be aspirated and the temperature of the incubator. These settings can be carried out using arrows' buttons.
The following picture shows the Body Windows for the wavelength setting, for the other two features the Body Window will appear similar:

```
Read filter    (nm)

340
```

Use arrows to select the filter, then press ENTER to confirm and go to the next setting. On the contrary press STOP to exit from ABS mode. When you completed the settings, on the Body Window will appear the message "INSERT BLANK". Insert the blank cuvette into the reading cell and press ENTER. Then will appear the message "INSERT SAMPLE": insert the sample cuvette into the reading cell and press ENTER. The absorbance value will continuously be shown until STOP key is pressed. This method, applied to sample control solutions with known absorbance value at a specific wavelength,can be used to verify the instrument accuracy.

4.2) Pump calibration

The peristaltic pump guarantees the maximum precision in the intake volume of the liquid (or air) into the flow cell. The pump must be calibrated when the instrument is used for the first time and also periodically. The calibration of the intake system can be carried out following this procedure: in the main menu press MENU button and select “SERVICE”.
You will reach Service Menu and the Body Window appears as explained above.
Select “PUMP CALIBRATION” and then press ENTER: the script “EXECUTING…” will appear in a window in the middle of the display. Place a cuvette containing exactly 5 ml of distilled water under the intake spout. Press the sample lever. The pump is now in action and draws the liquid from this cuvette (make sure it does not draw air). When the pump has drawn all the liquid, press again the sample lever. Intake stops and on the display will appear, only for a few seconds, a number. Such a number indicates, in ms, the time necessary for the intake of 5 ml of liquid. The instrument now returns automatically into the main menu.

4.3) Diagnostic & Service

You can reach “Diagnostic & Service” selecting “Diagnost. & Service” in the Service Menu. The Body Window will appear as follows:

```
Quick Diagnostic

Service Only
```

If you select “QUICK DIAGNOSTIC” the analyser will print some self-diagnostic parameters, which could be helpful when you call service to solve any problem.
Do not ever select “SERVICE ONLY”, this area has access reserved for service engineer. If for mistake you access to this area, you can safely turn off the instrument and turn it on again.

5 PRINTING OPERATIONS

5.1) Printing and display of the analyses' name list

When the display shows the Main Menu (see Fig. 6), press MENU button and then select "PRINT". The Body Window appears as follows:

```
Test list

Result Printout

Test Parameter
```

Select Test List using UP and DOWN arrows' buttons and then press ENTER. The instrument prints the analyses' name list. During the printing step you can press STOP to interrupt the operation.

5.2) Printing of analytic parameters of one analysis

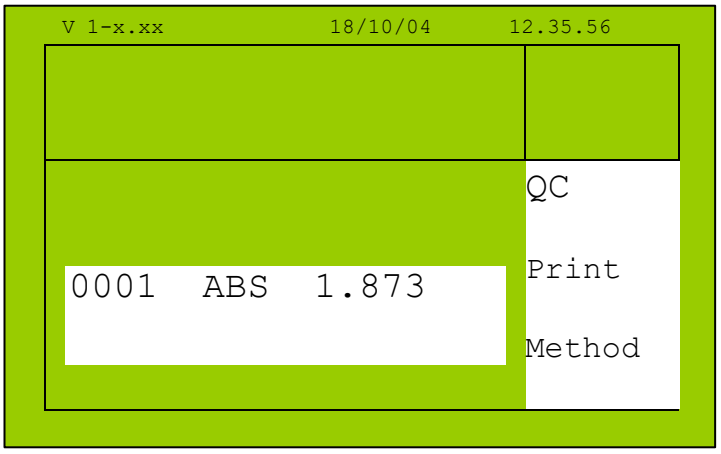
It is possible to print the list of parameters of all the stored analyses as follows. The parameters will be printed in partial or total way, depending on the printer set-up. In the Main Menu press MENU and then select "PRINT". The Body Window will have this feature:

```
Test List

Result Printout

Test Parameter
```

Select "TEST PARMETER", then choose the analysis and press ENTER: the instrument will print the parameter of the choosen test.

5.3) Printing of results

The results of an analysis can be printed automatically (see "SETTING" menu par. 2.5) at the end of the analysis. Otherwise, as the display shows the result (see the picture below as example):

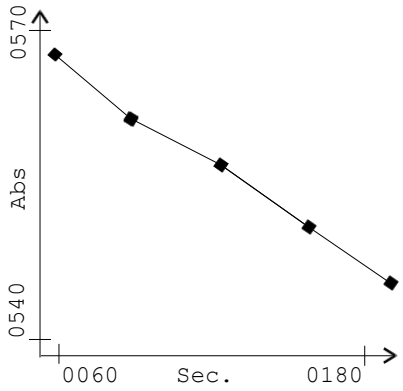


press MENU button to shift the cursor in the Menu Window and select “PRINT”. Press ENTER and wait the end of printing operation.
The printed result contains the following information (common to all analyses):

- Progressive number (1 to 9999)
- Measuring unit
- Value
- Indication of exceeding the preset limit values of the analysis. In case the result is less than the lower limit value, the letter **L** appears on the right side of the result. In case the result exceeds the higher limit value, the letter **H** appears this time.
- Indication of exceeding linearity limits of the test. In this case you see, the letter **D** on the right side of the result
- Indication that the analysis has been done at a temperature which is different from the programmed one. In this case, you see the symbol * at the right side of the result.

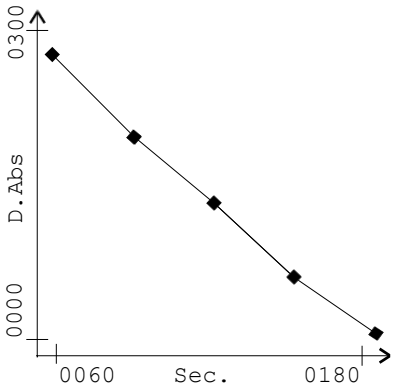
In kinetic and multistandard analyses is also possible to plot a graphic chart. In the figure below an example is given. Both these two graphics were obtained from kinetic analyses.

```
Abs 0.....0564
D.Abs.....0008
D.Abs.....0006
D.Abs.....0007
D.Abs.....0006
```




```
0010     U/L 0042
```

```
D.Abs.....0008
D.Abs.....0006
D.Abs.....0007
D.Abs.....0006
```




```
0007     U/L 0056
```

5.4) Printing result memory

The result memory can be printed out by selecting “RESULT PRINTOUT” in the “PRINT” Menu. In this program is possible to select a printout type (by ID, by DATE, by TEST).

The Body Window will show:

```
Search by ID Nr

Search by test Nr

Search by date
```

Select the printout type using the arrows’ buttons and pressing ENTER.
(See paragraph 6.11 for further details)

6 CALCULATION PROCEDURES PERFORMED BY 3000 EVOLUTION

6.1) *End-point* analysis

The instrument measures the sample ABSORBANCE, multiplies it by a factor (K) and displays the results directly in the programmed concentration units. The calculation factor can be entered directly or obtained bythe instrument if a standard is used. In this case the factor is obtained by the formula:

$$K = \frac{C_{STD}}{ABS_{STD}}$$

C_{std} = value of the standard used
ABS_{std} = absorbance of standard

Such, K-factor value then is used for the calculation of sample concentration as follows:

$$C_{SAMPLE} = ABS_{SAMPLE} \cdot K$$

C_{SAMPLE} = Sample concentration

ABS_{SAMPLE} = Sample Absorbance

6.2) *Kinetic* analysis

In a kinetic analysis the reaction speed is determined by taking series of measurements of sample ABSORBANCE.
The instrument begins to read after a preset wait time (*delay*). It reads continuously during the *reaction time* and calculates as many absorbance variations as the set number of readings (reading nr). The instrument then calculates the ABSORBANCE difference between one measurement and the previous one (ABS) and determines the average value, referred to 1 minute (D ABS/min.). Therefore, the following relation is valid:

Enzymatic activity (U/L) = D ABS/min. x K

Remember that K, usually indicated by the reagent manufacturer, can be obtained from the following formula:

$$K_{FACTOR} = \frac{V_{tot} \cdot 1000}{V_{sample} \cdot e \cdot s}$$

Vtot = Total volume of the reaction mixture

Vsample= Sample volume
e = Molar extinction coefficient of chromogen
s = Cuvette thickness (in cm)

6.3) *Fixed-time* analysis

In this operating mode the instrument does two ABS measurements on the sample being examined:

- The first one after a *delay* time from the moment ENTER button is pressed.

- The second one after a *reaction time* from the first reading.

The instrument calculates the Absorbance difference between both readings (ABS). The concentration

calculation is obtained by multiplying the ABS by a suitable K. This K can be:

1) entered directly by the operator (*Fixed-time* with K)
2) calculated by the instrument, using a standard (*Fixed-time* with standard)

Such calculation procedure is similar to the one used for *End-point* analyses.

6.4) *Multistandard* analysis

In this operating mode the instrument does a linear interpolation between the various points of the calibration curve; the instrument can interpolate up to 7 calibration points. Starting from the interval in whom the absorbance value of sample is, the result is given by the following formula:

$$C_{SAMPLE} = C_n + \frac{(C_{n+1} - C_n) \cdot (ABS_{SAMPLE} - ABS_n)}{ABS_{n+1} - ABS_n}$$

$\mathbf{C}_{sample}$= Sample concentration
$\mathbf{C}_{n}$= Concentration of standard n
$\mathbf{C}_{n+1}$= Concentration of standard n+1
$\mathbf{ABS}_{sample}$= Sample absorbance
$\mathbf{ABS}_{n}$= Absorbance of standard n
$\mathbf{ABS}_{n+1}$= Absorbance of standard n+1

n is the number of the standard according to the following relations:

$\mathbf{ABS}_n < \mathbf{ABS}_{sample} < \mathbf{ABS}_{n+1}$

6.5) Absorbance calculation when bichromatism is used

For the *End-point* and *multistandard methods*, the reading can be done by using bichromatism; in this case the calculation procedure is the same as described before, the only parameter which varies is the absorbance calculation, according to the following formula:

$\mathbf{ABS} = \mathbf{ABS}_{let} - \mathbf{ABS}_{bic}$ $\mathbf{ABS}$ = Absorbance value
$\mathbf{ABS}_{let}$ = Absorbance of reading filter
$\mathbf{ABS}_{bic}$ = Absorbance of bichromatism filter

6.6) Differential

This kind of analysis is performed as an End-Point analysis, but the flag REPEAT BLANK is set to YES.
The sample's concentration is calculated according to the following formula:

$$C_{SAMPLE} = (ABS_{R1+R2+SAMPLE} - ABS_{R1+SAMPLE}) \cdot K_{FACTOR}$$

$\mathbf{ABS}_{R1+R2+SAMPLE}$ = Absorbance value of sample plus reagent 1 and reagent 2
$\mathbf{ABS}_{R1+SAMPLE}$ = Absorbance value of sample plus reagent 1
$\mathbf{C}_{SAMPLE}$ = Sample concentration
$\mathbf{K}_{FACTOR}$ is calculated with the following formula:

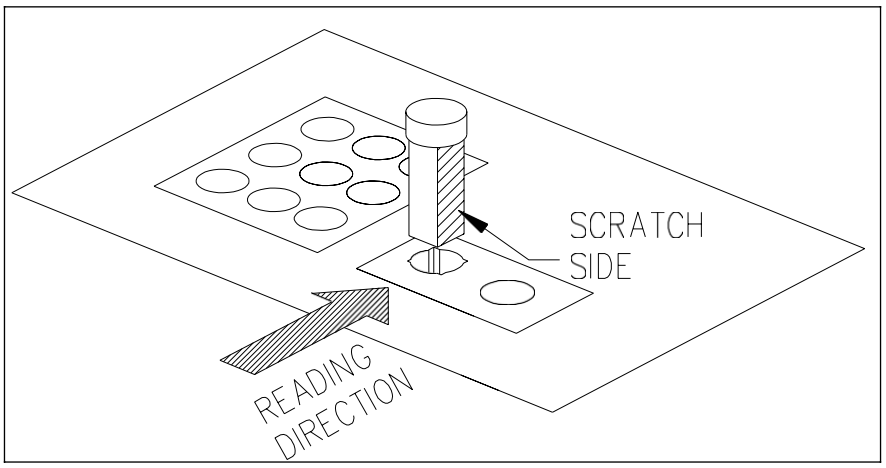
$$K_{FACTOR} = \frac{C_{STANDARD}}{ABS_{R1+R2+STANDARD} - ABS_{R1+STANDARD}}$$

$\mathbf{C}_{STANDARD}$ = Standard sample concentration
$\mathbf{ABS}_{R1+R2+STANDARD}$ = Absorbance value of standard sample plus reagent 1 and reagent 2
$\mathbf{ABS}_{R1+STANDARD}$ = Absorbance value of standard sample plus reagent 1

7 EXECUTION OF ANALYSES

Disposable cuvettes, commonly used in laboratory, as well as the flow cell cuvette can be operated on this instrument. Optical readings using laboratory cuvettes are handled by the keyboard (ENTER key). To read a flow cell, you must only press the sample lever (PUSH button). In all the following examples, we have only explained reading procedures using the keyboard in order to summarize.

NOTE: If cuvettes are used, insert the cuvette in the analyzer with FLAT face towards the operator and use ENTER key on keyboard to perform reading.



WARNING: ALWAYS WEAR GLOVES WHEN HANDLING CUVETTES, REAGENTS AND ALSO WHEN CLEANING THE INSTRUMENT

7.1) Absorbance readings (ABS mode)

Plain absorbance readings can be determined entering in the service menu.See paragraph 3.1 for further details.

NOTE: DEFECTIVE FILTERS OR LAMP WILL LEAD TO UNCORRECT MEASURES. ANALYSER WARN USER IN CASE OF ANOMALIES.

7.2) Selection of the analysis

When switching on the instrument, after self-test completion, the display shows the Main Menu (Fig. 6): you can select the analysis using arrows' buttons, then press ENTER. With UP and DOWN you can scroll the analysis menu in the current page, with LEFT and RIGHT arrows you will scroll the analysis menu page by page (the 120 test are distribuited in 20 pages). Once an analysis is selected, its parameters are printed (if automatic printing has been enabled). On the display, in the Top Window, the first line indicates the name of the analysis, the analysis kind, the symbol K and the constant K value, the second line shows the Low limit Value and the High Limit Value with the correct unit.

7.3) Execution of an *End-point* analysis with K

Select the desired analysis as explained in paragraph 6.2
On the Body Window will appear the message "INSERT BLANK". Insert Blank cuvette in the reading cell and press ENTER. You can skip this step if "BLANK SAVE" is enabled (see chapter 9 for details).
Then, as the message "INSERT SAMPLE" appears on the Body Window, insert the sample cuvette in the reading cell and press ENTER. The analyzer will wait few seconds before reading the sample. The result of the analysis is then displayed and, if automatic printing is preset, also printed. The progressive sample number, the absorbance value expressed in mAbs units, the measuring unit and the digital value appear on the display.
Next picture shows the appearance of Body Window after the calculation is performed:

```
ID: 0003 mAbs: 0658


018.2    g/dL
```

7.4) Execution of an *End-point* analysis using standard

Select the desired analysis as explained in paragraph 5.2.
On the Body Window will appear the message "INSERT BLANK": insert the blank cuvette into the reading cell and press ENTER. You can skip this step if "BLANK SAVE" option is enabled (see chapter 9 for further details).
After this step, the Body Window will appear as follows:

```
Calibration?

Yes
```

Using LEFT and RIGHT arrows to choose if you want to perform a new calibration. If you select NO the instrument uses the K-factor calculated in the last calibration for this test, if you select YES the analyser will

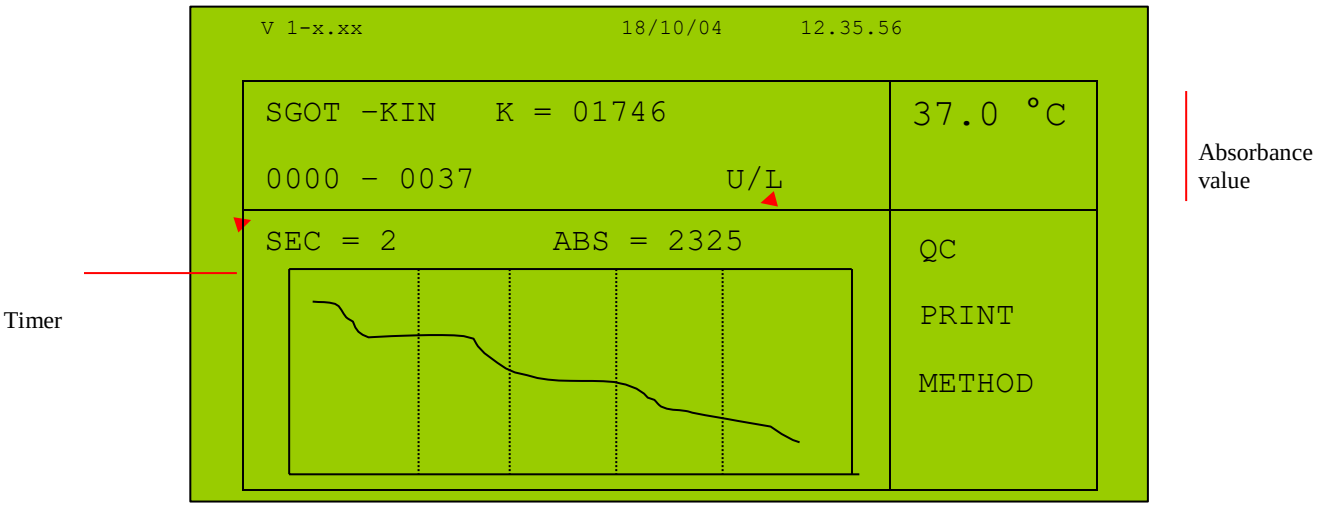
perform the calibration. As the message “INSERT STANDARD” appears on the Body Window, insert the cuvette into the reading cell and press ENTER.
The instrument determines the new K and updates its value on the display (Top Window). Then on the Body Window will show the message “INSERT SAMPLE”: insert the sample cuvette in the reading cell and press ENTER.
After reading the sample, the result of the analysis is shown on the display and, if automatic printing is preset, the result is also printed. The progressive sample number, the absorbance value expressed in mAbs units, the measuring unit and the digital value appear on the display as shown in paragraph 6.3.

7.5) Execution of a *Multistandard* analysis

Analysis between 51 and 120 are open and can also be used for multistandard-type analyses.
In this case, the operator must program them correctly (see chapter 7 for further details) before he can perform them. After the analysis has been correctly programmed, select the test as explained in paragraph 5.2.
As the message “INSERT BLANK” appears on the Body Window, insert the blank cuvette into the reading cell and press ENTER. You can also skip this step if BLANK SAVE is enabled (see chapter 9 or further details). Then the message “INSERT STANDARD 1” will appear on the display: insert the standard 1 cuvette into the reading cell and press ENTER. Repeat this operations for each standard required by the analysis. The number of standards is chosen in the analysis programmation (see chapter 7). After the last standard cuvette required, the instrument requests the sample cuvette displaying “INSERT SAMPLE” on the Body Window: insert the sample cuvette into the reading cell and press ENTER.
The instrument executes a linear interpolation between each pair of concentration values of the various standards. The result is shown on the display as explained in paragraph 6.3. If the concentration of the sample is less than that of the lowest standard, LLL is shown on the display. On the contrary, if the concentration of the sample is greater than that of the highest standard, HHH is shown on the display: in this case, dilute the sample and repeat the analysis.

7.6) Execution of a *Kinetic* analysis

Select the desired analysis as explained in paragraph 5.2.
On the Body Window appears the message “INSERT SAMPLE XXXX”: insert the sample cuvette and press ENTER. While the machine executes the test, the display draws the graphic of the kinetic of the reaction, as shown in the following picture:



The timer indicates the residual time (in seconds) to complete the analysis. When the sample analysis is at the end the result is visualized on the display; if automatic printing is preset, this result is also printed. The display shows the absorbance

value read at second 0 of reading interval, the absorbance variation referred to 1minute (D ABS/min) and the result with correct measure unit as shown in next picture:

```
Abs 0 ..... 1979

Abs/m ..... 000.6

0.125          U/L
```

Particular of Body Window of the display

7.7) Execution of a *Fixed-time* analysis with K

Select the desired analysis as explained in paragraph 5.2.
As on the Body Window appears the message “INSERT SAMPLE XXXX”, insert the sample cuvette into the reading cell and press ENTER The test will start and the display will show the graphic as in Kinetic analysis.
The timer indicates the residual time (in seconds) to complete the analysis. When the sample analysis has been completed the result is visualized on the display; if automatic printing is preset, this result is also printed. The progressive number, the measuring unit and the analytical result appear on the display.

7.8) Execution of a *Fixed-time* analysis with standard

Select the desired analysis as explained in paragraph 5.2.
The instrument asks to you if you want to perform a new calibration: on the Body Window appears the following message

```
Calibration?

YES
```

Use LEFT and RIGHT arrows to select YES or NO. If you select NO, the instrument will use the K-factor calculated in the last calibration for this test. If you select YES the message “INSERT STANDARD” will appear on the Body Window: insert the standard cuvette and press ENTER.
The timer on the display the residual time (in seconds) to achieve the calibration. When the timer indicates 0, the calibration is completed. The instrument determines the new K and shows its value on the display.
Then on the Body Window will appear the message “INSERT SAMPLE”: insert the sample cuvette and press ENTER. The test will start and the display will show the graphic as in Kinetic analysis.
The timer indicates the residual time (in seconds) to complete the analysis. When the timer indicates 0, the sample analysis is completed and the result is visualized on the display; if automatic printing is preset, this result is also printed. The progressive sample number, the measuring unit and the analytical value appear on the display.

7.9) Reading at incubator temperature different from the programmed one

If you carry out an analysis at a temperature which is different from the one entered in the program, “No temp” will appear instead of “temp OK”. An asterisk (*) will be printed beside the analytical result.

7.10) Modifying the progressive number

The progressive number is a four-digit number that increases automatically by 1 unit whenever an analysis is carried out. This number is displayed and printed as the first datum before the measuring unit.
This value can be modified when one of the following messages appears on the second Body Window:

“INSERT BLANK”
“INSERT SAMPLE XXXX”
“INSERT STANDARD”
Use arrow keys to modify ID number

Every time the user is performing a test the instrument is storing in memory the result of the test with the corresponding ID number. For this reason, every time the user is asked of inserting a sample, the instrument will automatically display also the corresponding ID number:
The procedure is a bit different if you are reading or not using the flow cell.

USING FLOW CELL:

```
Insert sample 0001
```

When on the Body Window appears this message, the analyser is ready to read and will associate the next result with the ID Number displayed (0001 in the example). Press PUSH button: the display will show the ABS result acquired (for example 1.894)

```
0001          ABS        1.894
```

The result is now stored to the corresponding ID number (in the example 0001). The instrument is ready to executes another reading, and will associates the next result to ID number 0002, if you execute it. If you want to change the ID Number of next test, you have to do it before executing it. To do it, press ENTER **2 times consecutively**: on the Body Window will appear the following two messages:

```
Insert sample 0002
```

And then:

```
Insert ID Number
0002
```

Using LEFT and RIGHT arrows you will select the digit to change, using UP and DOWN arrows you will change the digit. When the ID Number is the one you want, press ENTER to continue reading.

USING CUVETTES:

```
Insert Sample 0001
```

When the Body Window show this message, the analyser is ready to read and will associates the result with the ID number displayed (0001 in the example). Press ENTER to read cuvette: the display will show

```
0001          ABS          1.894
```

Press ENTER: the display will show:

```
Insert Sample 0002
```

If you want to change ID number, just use LEFT and RIGHT arrows to select the digit to change and UP and DOWN arrows to change the digit. Then press ENTER to continue reading.

7.11) Recalling memory result

The result memory can be printed out by selecting “Result Printout” in the “PRINT” Menu (see paragraph 3.5). In this program is possible to select a printout type (by ID, by DATE, by TEST).

The Body Window will show:

```
Search by ID Nr

Search by Test Nr

Search by Date
```

If you select “SEARCH BY ID NUMBER” the display will show:

```
Search by ID Nr

------
```

Use LEFT and RIGHT arrows to select the digit, UP and DOWN arrows to change the digit and ENTER to confirm. Then on the display will appear this message:

```
Set Date :
18/10/2004
```

Set the date using arrows’ buttons and confirm pressing ENTER for each digit. Then the display will show the last message:

```
Set Name :
```

Use again arrows’ buttons to set a Name. The machine will print out the tests’ results corresponding to your selection.

If you select “SEARCH BY TEST NR” you have to follow the same procedure as “SEARCH BY ID NUMBER” but software will not ask you to set a Name.

If you select “SEARCH BY DATE” you have only to select the date: the analyser will print out all the tests’ results executed on the chosen date.

7.12) Waste processing

Always follow the common clinical laboratories rules to process the waste bottle content, exhaust reagents, used cuvettes and any other object that may be contaminated with organic and/or chemical fluids.

8 ANALYSES PARAMETERS

Analyses' parameters refer to all information required by the instrument to carry out that analysis according to the preset methods. The following table shows in a schematic way all the necessary parameters to be entered for each type of analysis procedure.

Parameter	*End-point* (EP) with K	*End-point* (EP) with standard	*Kinetic* (KIN)	*Fixed-time* (FXT) with K	*Fixed-time* (FXT) with standard	*Multistandard*
Method type	×	×	×	×	×	×
Method name	×	×	×	×	×	×
K	×		×	×		
Standard concentration	×	×	×	×	×	×
Read Filter	×	×	×	×	×	×
Bichromatic Filter	×	×				×
Measuring Units	×	×	×	×	×	×
Decimal point	×	×	×	×	×	×
Working temperature	×	×	×	×	×	×
Initial Delay	×	×	×	×	×	×
Reaction time			×	×	×	
Number of readings			×			
Standard number						×
Repeat blank	×	×				×
Use blank			×	×	×	
Low limit	×	×	×	×	×	×
High limit	×	×	×	×	×	×
Linearity limit	×	×	×	×	×	×
Sample volume	×	×	×	×	×	×
First reagent volume	×	×	×	×	×	×
Second reagent volume	×	×	×	×	×	×
Intake volume	×	×	×	×	×	×

8.1) Bichromatic filter

In End-point methods (using K or standard) and multistandard ones, it is possible to perform the optical measurement using the bichromatic filter as well as the reading one. The use of such filter reduces the optical background noise in the chemical reaction. The instrument measures the absorbance of the reading filter at the corresponding wavelength and that of the bichromatic filter at another wavelength. It determines the difference between these two absorbance values and gives the final result.

The chromogen formed in the reaction must not absorb at the wavelength selected for the bichromatic filter. In this way, the background noise can be subtracted from the optical determination; such a noise is usually constant at all wavelengths.

8.2) How to use the decimal points

Depending on the number of decimal points you want to express the result with, you must insert the k-factor and the standard value in the following way:

K-Factor Of the method	Limit (low , high or linearity) of method	Decimal points	K-factor to be entered	Limit to be entered	Result
1745	51	0	1745	51	45
1745	51	1	17450	510	45.0
Standard used in method	Limit (low , high or linearity) of method	Decimal points	Standard to be entered	Limit to be entered	Result
8	15	0	8	15	8
8	15	1	80	150	8.0
8	15	2	800	1500	8.00
75	160	0	75	160	75
75	160	1	750	1600	75.0
2.7	6	1	27	60	2.7
2.7	6	2	270	600	2.70

8.3) Programming Flow Cell aspiration volume

The sample aspiration volume can be programmed separately for each test. This volume depends mostly from the kit manufacturer and the flow cell you are using. For instance, if you use a 18 µl flow cell you can work with aspirated volumes that are less than those needed when using a 80 µl flow cell.

To program the sample aspiration volume intake by flow cell select the analysis you wish to modify and follow the instruction explained in "MODIFYING THE PARAMETERS AND PROGRAMMING A NEW ANALYSIS" (chapter 8), selecting the parameter: "sample aspiration".

When choosing the aspiration volume please refer to test kits manufacturer and to their recommended volume. **Typical working volume is 500 µl.**

It is however possible to reduce the aspiration volume in order to save reagent by using air-gap. Please refer to APPENDIX D: REDUCING CARRY-OVER AND WORKING VOLUME USING AIR-GAP for this procedure.

9 MODIFYING THE PARAMETERS AND PROGRAMMING A NEW ANALYSIS

To modify the parameters of an analysis, you must first select the analysis in the Main Menu using arrows' buttons and pressing ENTER. In the Body Window will appear one of the following message: "INSERT BLANK", "INSERTSAMPLE", "INSERT STANDARD", "INSERT TEST". Press MENU to interact with Menu Window and select "METHOD". The Body Window appears as follows:

```
Type Method

Parameter Method
```

Select "PARAMETER METHOD" and press ENTER. Use UP and DOWN arrows to slide the menu and ENTER to select the parameter which has to be modified. Use arrows' buttons to modify the values then press STOP. After you have modified all the parameters you wanted, press STOP and then ENTER to save changes in memory.

The table below shows a schematic of the parameters that can be present in each method. These parameters can change according to the type of method (for example, in EP method there will not be reaction time, while it will be present for KIN test).

Parameters **(inserted data)**	***End-Point*** **method (EP) with K**	***End-Point*** **method (EP) with standard**
Method **(EP, KIN, FXT, MSD)**	Select EP using arrows and pressing ENTER.	
New name **(any abbreviation by letters or numbers)**	Write the new abbreviation using arrows and pressing ENTER.	
Use standard **(YES/NO)**	Press STOP	Press ENTER
New Factor **(1-59999)**	Enter the new value of the k-factor using the arrows. Such value must be entered without decimal points	NOT REQUIRED
Standard **(1-9999)**	NOT REQUIRED	Enter the concentration value of the new standard using arrows.
Read filter **(340, 405, 492, 505, 546, 578, 630, ( ))**	Select the reading wavelength using arrows' buttons and pressing ENTER. ( ) corresponds to empty position.	
Bic. Filter **(340, 405, 492, 505, 546, 578,630, ---, ( ))**	Select the bichromatic wavelength using arrows and pressing ENTER. To disconnect the bichromatic reading select ----. ( ) corresponds to empty position.	
Units **(see list in table par. 10)**	Select the measuring unit of the results using arrows and pressing ENTER.	
Decimal points **(0, 1, 2)**	Select the numbers of decimal point of the result using arrows and pressing ENTER.	
Temperature **(---, 20° to 40°)**	Select the thermostat temperature by pressing ENTER. To disconnect the thermostat select 00. Values lower then 20 °C or greater than 40 °C are not admitted	
Delay **(0-999)**	Enter the wait-time in seconds, before the final reading using arrows.	
Repeat blank **(YES/NO)**	Select YES if the method requires blank repeating for each sample. If not, select NO.	
Lower Limit **(0-9999)**	Enter the lower reference value of this parameter using arrows.	
Higher Limit. **(0-9999)**	Enter the higher reference value of this parameter using arrows.	
Linearity Limit. **(0-9999)**	Enter the higher linearity limit of the reagent to be used using arrows.	
Volume SM **(0-999)**	Enter the sample volume in µL as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reaction.	
Volume Rea1 **(0-9999)**	Enter the volume of reagent 1, in µL, as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reaction.	
Volume Rea2 **(0-999)**	Enter the volume of reagent 2, in µL, as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reactions	

Asp. Volume **(0-9999)**	Enter the intake volume of the reagent into the flow cell using arrows.

Parameters **(possible data)**	***Fixed-time*** **Method (FXT) with K**	***Fixed-time*** **Method (FXT) with standard**	***Kinetic*** **(KIN) Method**
Method **(EP, FXT, KIN, MSD)**	Select FXT using arrows and pressing ENTER	Select FXT using arrows and pressing ENTER	Select KIN usin arrows and pressing ENTER
New name **(any abbreviation by letters or numbers)**	Write the new abbreviation using arrows' buttons and pressing ENTER		
Use Standard **(YES, NO)**	Select NO	Select YES	NOT REQUIRED
New factor **(1-59999)**	Enter the new k-factor value using arrows. Such value must be entered without decimal points.	NOT REQUIRED	Enter the new k-factor value using arrows. Such value must be entered without decimal points.
Standard **(1-9999)**	NOT REQUIRED	Enter the concentration value of the new standard using arrows. Such value must be entered without decimal points.	NOT REQUIRED
Read filter **(340,405, 492, 505, 546, 578, 630, ( ))**	Select the reading wavelength using arrows and pressing ENTER. ( ) corresponds to empty position.		
Unit **(see list in table par. 10)**	Select the measuring unit of the results using arrows and pressing ENTER.		
Decimal points **(0, 1, 2)**	Select the numbers of decimal point of the result using arrows and pressing ENTER.		
Temperature **(---, 20° to 40°)**	Select the thermostat temperature using arrows and pressing ENTER . To disconnect the thermostat select 00. Values lower than 20 °C or greater than 40 °C are not admitted		
Delay **(0-999)**	Enter the time in seconds from the moment you start the analysis until the first reading, using arrows and pressing ENTER.		
Reaction T **(1-999)**	Enter the time in seconds between the first and the second readings by pressing arrows' buttons		Enter the total time in seconds between the first and the last readings using arrows
Reading nr (1-9)	NOT REQUIRED	NOT REQUIRED	Enter the number of readings you want the instrument to perform, using arrows. Note that if you select a number of readings of 5, you will obtain 5 absorbance variations.
Use Blank **(YES, NO)**	Select YES if blanking is needed. If not, select NO.		
Lower Limit **(0-9999)**	Enter the lower reference value of this parameter using arrows.		
Higher Limit. **(0-9999)**	Enter the higher reference value of this parameter using arrows.		
Linearity Limit. **(0-9999)**	Enter the higher linearity limit of the reagent to be used using arrows.		
Volume SM **(0-999)**	Enter the sample volume in µL as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reactions		
Volume Rea1 **(0-9999)**	Enter the volume of reagent 1, in µL, as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reaction		
Volume Rea2 **(0-999)**	Enter the volume of reagent 2, in µL, as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reaction		
Asp. Volume **(0-9999)**	Enter the intake volume of the reagent into the flow cell using arrows.		

***P**arameters* **(possible data)**	***Multistandard* Method (can be programmed from channel 51 to 120)**
Method **(EP, KIN, FXT, MSD)**	Select MSD using arrows. Then press ENTER.
STD number **(1,2,3,4,5,6,7)**	Enter the number of standards to be used to plot the calibration curve using arrows and pressing ENTER. You can enter up to number 7.
Standard 1 **(1-9999)**	Enter the concentration value of the first standard. The standard must be introduced in an increasing order of concentration values.
Standard 2 **(1-9999)**	Enter the concentration value of the second standard. The standard must be introduced in an increasing order of concentration values.
Standard 3 **(1-9999)**	Enter the concentration value of the third standard. The standard must be introduced in an increasing order of concentration values.
Standard 4 **(1-9999)**	Enter the concentration value of the fourth standard, etc. The standard must be introduced in an increasing order of concentration values.
New name **(any abbreviation, by letters or numbers)**	Write the new abbreviation using arrows and pressing ENTER.
Read filter **(340, 405, 492, 505, 546, 578, 630, ( ))**	Select the reading wavelength using arrows and pressing ENTER. ( ) correspond to empty position
Bic. Filter **(340, 405, 492, 505, 546, 578, 630, ---, ( ))**	Select the bichromatic wavelength using arrows and pressing ENTER. To disconnect bichromatic reading select ---. ( ) correspond to empty position
Units **(see list in table par. 10)**	Select the measuring unit of the results using arrows and pressing ENTER.
Decimal points **(0, 1, 2)**	Select the numbers of decimal point of the result using arrows and pressing ENTER.
Temperature **(---, 20 °C to 40 °C)**	Select the thermostat temperature using arrows and pressing ENTER. To disconnect the thermostat select 00. Values lower than 20 °C or greater than 40 °C are not admitted
Delay **(0-999)**	Enter the wait-time in seconds, before the final reading using arrows.
Repeat BLK **(YES, NO)**	Select YES if the method requires blank repeating for each sample. If not, select NO.
Lower Limit **(0-9999)**	Enter the lower reference value of this parameter using arrows.
Higher Limit. **(0-9999)**	Enter the higher reference value of this parameter using arrows.
Linearity Limit. **(0-9999)**	Enter the higher linearity limit of the reagent to be used using arrows.
Volume SM **(0-999)**	Enter the sample volume in µL as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reaction
Volume Rea1 **(0-9999)**	Enter the volume of reagent 1, in µL, as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reaction
Volume Rea2 **(0-999)**	Enter the volume of reagent 2, in µL, as stated in the method using arrows. This is a memo parameter. It is not used by the instrument at all. It can however be printed to summarize the conditions of the reaction

Asp. Volume **(0-9999)**	Enter the intake volume of the reagent into the flow cell using arrows.

10 QUALITY CONTROL

10.1) Quality Control program

For all methods it is possible to have up to 2 quality control program for each channel, for a maximum number of 30 total independent quality control programs.

The quality control collects the last 30 results, and calculates (after a complete acquiring of the 30 samples) the more important statistical parameters, such as :

Mean = Mean Value
SD = Standard Deviation
CV = Coefficient of variation

With these parameters analyzer can print Levey-Jennings control chart, with 2s and 3s interval. The acquired result can be recalled at any time. See section below for more details.

10.2) How to enable/disable Quality Control program for the current test

In each test which is performed inside the cuvette section (cuvette or flow cell) can be enable/disable the QC program. For each test it is possible to enable 2 different QC programs, with the limitation that the total numbers of enabled QC program cannot exceed 30.
To enable or disable the QC program for a test, select the test and enter in Method Menu to select "PARAMETER METHOD" as explained in Chapter 7. Scroll the Menu until you reach "ENABLE QC1" or "ENABLE QC2".

Important:
Disabling QC and saving the changes will erase the existing QC memory, loosing the QC parameters and data.

10.3) How to collect a QC sample

Select the test for which you want to collect a QC sample in the Main Menu using arrows' button and press ENTER
Press Menu to shift the cursor in the Menu Window, then select QC and press ENTER. The Body Window will appear as follows:

```
Set sample QC1

Set sample QC2

View history QC1

View history QC2
```

Select "SET SAMPLE QC1" or "SET SAMPLE QC2" according to the QC program for which you want to set the sample. As in the Body Window appear the message "INSERT QC", insert your QC sample and press ENTER or PUSH button (depending on whether you are using cuvette or flow cell). Once the sample has been read the analyzer will store it in the QC memory If the memory is full the instrument will ask you the following questions in the Body Window:

QC1 Shift data?

Answering YES you will tell the analyzer that the all samples inside the memory will be shifted of one step. The first sample is erased and the current sample is stored as the last sample acquired.
The examples below show this case.

SMP1 SMP2, SMP3, …. SMP29, SMP30 SMP31

◄ ◄

If you answer NO the instrument will ask you if you want to recalculate the statistical parameters:

QC1 Recalculate?

Answering YES you will tell the analyzer that the statistical parameters (such as MEAN, SD, CV and 2s, 3s interval) will be recalculated using last 30 parameters inside the memory as sample QC history.
If you answer NO the instrument will only ask you if you want to erase the QC memory, preserving the statistical parameters calculated in a previous session:

QC1 Erase?

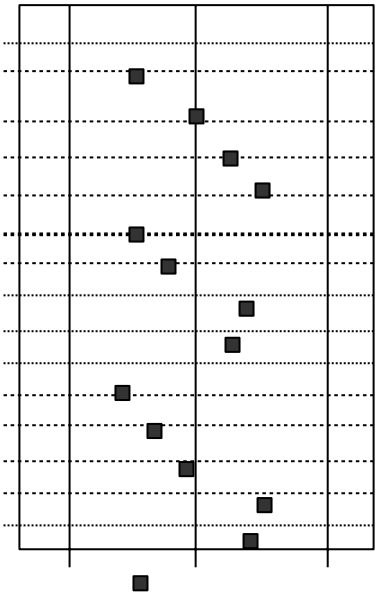
Answering YES you will tell the analyzer that the statistical parameters (such as MEAN, SD, CV and 2s, 3s interval) will remain the same and only the 30 QC sample data will be erased.

Note that example above refers to QC1 on an unspecified test. You will have the same behavior with each test and QC2.

10.4) Viewing QC data and graph

To access QC data you have to enter QC Menu as explained in paragraph 8.3 and then select “View history QC1” or “View history QC2”. If the QC memory is not empty, will be reported the data stored in the non volatile memory. If statistical parameters have already been calculated then the Levey-Jennings control chart it is also plotted (Fig. 7)

```
QC1
Units: U/L
Num. = 18
Mean = 03.34
SD   = 0.002
CV   = 1.4

15 Oct 08:00 3.30
16 Oct 08:04 3.38

. . .

12 Nov 08:03 3.34
14 Nov 08:09 3.34
```



-3S 2S +2S +3S

Figure 7: Levey-Jennings control chart

11 BLANK STORAGE FEATURES

The blank set is the storage inside the memory of the blank, in order to save reagent. The value of blank is permanently stored inside the memory of the instrument and used for absorbance and result calculation, until user decide to re-run a newblank or disable blank set.

The blank set can be enable or disable for each one of the 120 method of the instrument. To enable/disable this features go in “METHOD” Menu following the procedure explained in chapter 7. Then select “PARAMETER METHOD” and press ENTER. Slide the menu using UP and DOWN arrows’ buttons until you reach “BLANK SAVE” and press ENTER.

```
Blank Save

Yes
```

Select “YES” to enable or “NO” to disable blank set. If the blank set has been enable for a certain test (for example GLUCOSE), first time you will run this test instrument ask you for the REAGENT blank cuvette. A message will appear in the Body window:

```
Insert blank
```

As the blank has been read by the analyzer, it is stored inside the internal memory and used for calculations of result.

Every time you will enter a test where blank set has been enabled and blank has been already stored, the instrument will ask if the user want to recalibrate the blank (store a new blank inside the memory) with a message in the Body Window:

```
New Blank?

Yes
```

If you select “YES” the instrument will set a new blank into the memory, updating the old value with the new one. If you choose “NO” the instrument will continue using the old blank, allowing the user to save reagent.

NOTE 1:
Blank is linked to optical support used for executing test (flow cell or cuvette). Working with cuvette cannot recall a blank that was made in flow cell (and vice versa). If the working support changes for a certain test, a fresh blank has always to be performed on the new support.
NOTE 2:
Blank save features, to work properly needs a precise reference at startup. For this reason, analyser can't be switched on with cuvette left inside reading hole or (in flow cell mode) without having aspirated 1.5 mL of distilled water by PUSH button.

12 MULTISTANDARD EXTENDED MODE

The Multistandard test can work both as an End Point method with more than one standard (normal multistandard test) or as a Fixed time test with more than one standard. We call this mode is called “extended Multistandard mode” and it maybe useful for some fixed time test for whom calibration against one standard is not enough.
This mode it is enabled by enabling the Repeat Blank option in method editing menu and by setting a reaction time bigger than 0. Note that if you are no setting the reaction time, the test will behaves like a differential Multistandard test.

So according to the programmation you will have different possibilities:

Delay	≥ 0
Repeat Blk	NO
Reaction Time	*(hidden)*

Method:
End point test with more that one standard. If Delay is bigger than 0, the programmed delay time is wait before the reading

Delay	≥ 0
Repeat Blk	YES
Reaction Time	0

Method:
Differential test (sample blank) with more that one standard. Blank it is asked for each standard and sample. If Delay is bigger than 0, the programmed delay time is wait before the reading

Delay	> 0
Repeat Blk	YES
Reaction Time	> 0

Method:
Extended Multistandard mode. The test will be actually a Fixed Time Multistandard: after the reading command is given, the analyser will wait for the programmed Delay time, takes the first reading, wait for the programmed Reaction time, takes the second reading and calculates the delta absorbance as the positive difference between the 1st and the 2nd reading. In case of standard this value it is stored as standard absorbance for result calculation, and in case of samples it will be fitted to the calculated curve (with a linear interpolation, as explained in Multistandard result calculation), to get the concentration value.

13 TECHNICAL FEATURES

Optical system

Light source:	20 W long-life iodine incandescent lamp
Spectral field:	320 to 690 nm
Filter change:	automatic - motor-driven
Filters:	340, 405, 492, 505, 546, 578, 630 nm; 1 empty position
Detector:	solid state device

Thermostat

Heating element:	refrigerating and heating Peltier cell
Incubator Temperature:	selectable from 20°C to 40°C
Temperature accuracy:	±0.2°C
Stabilization period:	at least 15 min
Thermostatic unit:	10-position for square or cylindrical cuvettes, macro or semi-micro cuvette.

Flow system

Flow cell:	18 µL
Typical working volume:	500 µL
Minimum working volume:	350 µL
Carry over:	less than 1%
Intake:	peristaltic pump with programmable intake volume and air gap setting

Cuvette type 1cm optical-path square or cylindrical cuvettes

Measuring system:

Reset:	automatic
Measuring range:	-0.200 to +2.500 OD
Photometric linearity:	± 1% from 0 to 2.000 OD
Photometric accuracy:	± 1% from 0 to 2.000 OD
Precision:	CV < 1% @ 2.0 O.D
Reproducibility:	CV <1% from 0 to 2.000 O.D.
Drift:	lower than 0.005 OD per hour
Reagent volume in cuvette:	1 ml (minimum) for macro cuvette, 0.3 ml for semi-micro cuvette
Reagent volume in flow cell:	0.35 ml (minimum)
Wash function:	manual
Operating Modes:	Absorbance, End-Point, Kinetic, Fixed Time, Multistandard, Differential
Reading:	Monochromatic, Bichromatic

Data display and programming:

Keyboard:	8 Keys multifunctional keyboard or external connection for PS2 keyboard
Display:	Graphic 240 x 128 pixel
Thermal printer:	built-in graphic 24 columns high performance
Printer paper roll:	thermal type, 57mm wide, 44mm roll diameter.
Memory capacity:	120 programs
Reagent Blank Saving :	included
Results Memory:	400 test results
ID sample:	selectable
QC Program:	last 30 results, 2 levels for 30 selectable tests with Levey-Jennings Plot
Language:	English, Italian, 2 other language on request
Serial output:	RS-232 standard

General:

Power supply:	Auto sensing (80 – 260 V)
Dimension:	35x34x24Hcm

Weight:	11 kg
Working temperature:	15-30C°
Instrument class:	I
Installation class:	II

Serial transmission of data:

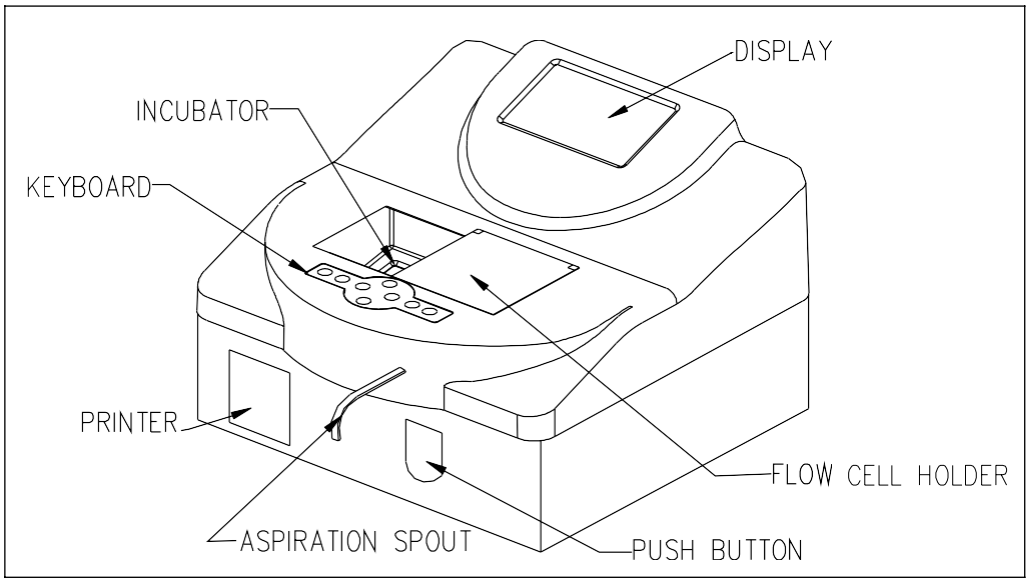
The serial output, standard type RS-232, uses the following transmission parameters: **38400, N, 8, 1.**
Refer to Appendix C for further details.

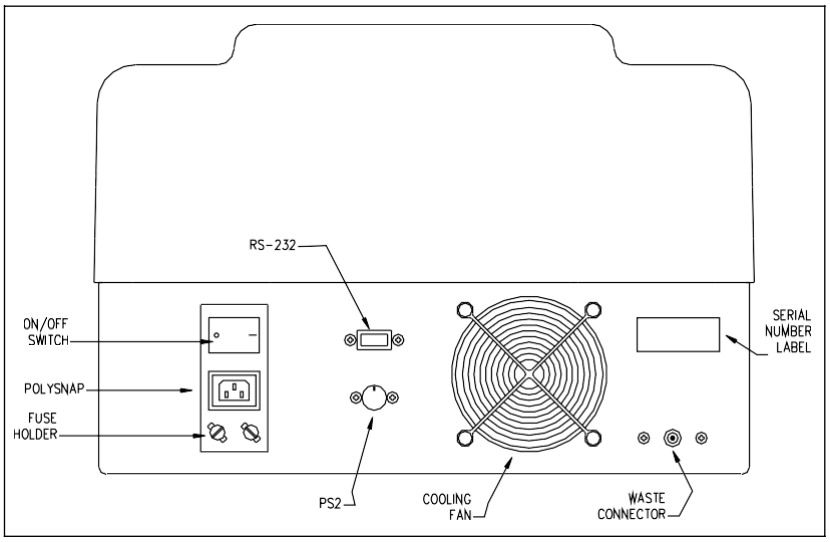
Serial connection:
The output connector, male 9-pole D-type, is located on the back of the instrument. The connections are as follows:

pin 2:	input
pin 3:	output
pin 5:	reference ground

To connect the serial input to a personal computer IBM type or IBM compatible, you can use a connecting cable directly into the three pins mentioned above.

14 DESCRIPTION OF MECHANICAL PARTS




RS-232
ON/OFF SWITCH
POLYSNAP
FUSE HOLDER
PS2
COOLING FAN
SERIAL NUMBER LABEL
WASTE CONNECTOR

APPENDIX A: TroubleShooting

This appendix shows the error messages related to issues that the user can normally solve by himself: if the problem persists, or a problem not listed below arise, contact your dealer.
Excluding the main plug fuses, **the instrument has no user serviceable parts: only trained technicians are allowed to service the instrument. An unauthorized action on the instrument may invalidate its safety and features, beside void the warranty.**
In case of suspect malfunctioning of the instrument, we recommend to check the instrument with colored solution or control serum of known value.
In the following table we describe messages and flags which appear on the Body Window or in a window which appears in the middle of the display:

MESSAGE	DESCRIPTION	POSSIBLE OPERATIONS
ERROR -1	The sample absorbance is too high or the reading is impossible, due to faulty instrument	Press ENTER to repeat the reading otherwise dilute the sample. If message persists call our service technicians.
Printer error	Error when printing	Press STOP to disconnect the printer, or any other key to try again.
Temperature Window is fixed	The thermostat is already regulated at the required temperature.	
Temperature Window is blinking	The thermostat has not yet reached the required temperature.	Wait.
Incorrect result when using blank saving	Incorrect result when using blank saving option.	Analyser has been switched on with a cuvette left inside or without having aspirated 1.5 mL of distilled water. Switch off analyser, switch it on again and initialize it correctly.
Incorrect result when using blank saving	Incorrect result when using blank saving option.	Ensure that the working mode (cuvette or flow cell) has not changed respect to last blank saved. If blank was saved using flow cell, can't be used for cuvette.
Printed occasionally: W_1_:xxx	Low energy on filter xxx	In most of the case analyser has been initialized incorreclty (with a cuvette left in reading hole or without a proper water aspiration of flow cell). Switch off analyser and repeat operation.
Persistent: W_1_CD:xxx	Low energy on filter xxx or lamp exausted	If this message do not disappears repeating the initialization procedure, try to recalibrate peristaltic pump (it could be due to an air bubble in to floe cell) or WASH flow cell. If not succeded, lamp or filter should be replaced.
Temperature Window shows - - - -	The temperature is disabled	
Const ERROR	Critical error in EEPROM	Call service.
EEP0 ERROR	Critical error in EEPROM	Call service
EEP1 ERROR	Critical error in EEPROM	Call service
EEP2 ERROR	Critical error in EEPROM	Call service
Delay XXX	XXXX = time (in seconds) required to complete the analysis.	Wait or press STOP to interrupt the analysis

- - -	In a *KIN* or *FXT* analysis, the absorbance-variation of the sample is too high during the first time-interval (Delay).	Dilute the sample.
LLLL	In an *MSD* analysis, the sample concentration is less than that of the lowest standard.	The sample concentration is lower than the minimum range of the method.
HHHH	In an *MSD* analysis, the sample concentration is greater than that of the highest standard	Dilute the sample.
CALIBRATE (Y/N)	It appears when you select an EP analysis with standard or FXT with standard. It allows the possibility to select a new calibration with the standard or read directly the sample cuvette	Press ENTER if a new calibration is required. The instrument will be ready to read the standard: INSERT STANDARD. Press STOP if a new calibration is not necessary. The instrument is ready to read the sample: INSERT SAMPLE.
D	The analytical result exceeds the linearity limit of the reagents.	Dilute the sample
*****	The thermostat has not reached the right temperature when the analysis was performed (blinking temperature window). The result therefore, is not correct.	Repeat the analysis when the thermostat and the reagents have reached the right temperature.
L	The analytical result is less than the lower limit value of the method.	
H	The analytical result is more than the higher limit value of the method.	

In the following table, we describe Error Messages which appear in the Error Message Area (see Fig. 1)

MESSAGE	DESCRIPTION	POSSIBLE OPERATION
TA ERROR	Error reading internal temperature	Call service
TW ERROR	Error reading incubator temperature	Call service
PRN PAPER	The roll of printing paper is over	Insert a new roll of printing paper
PRINT KO	The printer is disabled or malfunctioning	Disable printer or call service
PRN TEMP	Overheating of LPT	Wait
OVERHEATING	Instrument overheating.	Turn off and wait few minutes
H.FIL KO	Malfunctioning of filter positioning system	Call service

The instrument is provided with 2 fans. One fan is automatically controlled by microprocessor and it is switched on and off as required, according to the internal temperature of the instrument. Internal fan is always on and cool the incubation group.

APPENDIX B: Measuring Units ad Conversion factors

LIST OF MEASURING UNITS STORED IN THE INSTRUMENT

U/L	U/mL	mU/mL	mEq/L	MIL	mmo/L
7. µmo/L	8. nmo/L	9. %	10. g/L	11. mg/dL	12. µg/dL
13. g/dL	14. µg/mL	15. mg/L	16. ppm	17. F	18. NTU
19. units	20. Abs	21. µKat	22. Mi/µL	23. U/dL	

CONVERSION FACTORS OF ENZYMATIC ACTIVITIES (I.U.) AT DIFFERENT TEMPERATURES

ENZYME					
			25°C	**30°C**	**37°C**
ALAT (GPT)					
	at:	**25°C**	1	0.72	0.50
		30°C	1.39	1	0.69
		37°C	2.01	1.45	1
ALP					
	at:	**25°C**	1	0.77	0.57
		30°C	1.29	1	0.74
		37°C	1.74	1.35	1
α-AMYL					
	at:	**25°C**	1	0.81	0.65
		30°C	1.23	1	0.81
		37°C	1.53	1.24	1
ASAT (GOT)					
	at:	**25°C**	1	0.72	0.47
		30°C	1.39	1	0.65
		37°C	2.14	1.54	1
CK					
	at:	**25°C**	1	0.69	0.42
		30°C	1.27	1	0.60
		37°C	2.40	1.67	1
γ-GT					
	at:	**25°C**	1	0.73	
				0.56	
		30°C	1.37	1	0.77
		37°C	1.79	1.31	1
LDH					
	at:	**25°C**	1	0.69	0.42
		30°C	1.27	1	0.60
		37°C	2.40	1.67	1

EXTINCTION COEFFICIENTS ($cm^2/mole \times 10^6$)

	WAVELENGTH (nm)	**334**	**340**	**365**	**405**	**505**
CHROMOGEN						
NADH		6.18	6.3	1.33		
NADPH		6.18	6.3	1.33		

***p*-nitrophenol**	18.8	
Quinone imine		6.89

APPENDIX C : Serial TraNsmission protocol

Serial connector is on the back of the instrument, placed near the fan air flow aperture. See **10 Technical Features** for the pin used in the connection with a PC. The signal are according to the RS-232 standard.

Dyaset Srl provides on request a software to connect the instrument to a IBM compatiblecomputer: ask your dealer for details.

The protocol used to transmit data is the following:

Baud rate:	38400 Bps
Parity:	No
Data bit:	8
Stop bit:	1

Report Type:

After having selected and completed a test, PC will show following string as output:

```
JJJ NNNNNN IIII UUUUU MMMMM FFF
```

Where:

JJJ:	Number of the test
NNNNNN:	Name of the test
IIII:	Test ID
UUUUU:	Measure unit of the test
MMMMM:	Result of the test
FFF:	Flag bytes

APPENDIX D: REDUCING CARRY-OVER AND WORKING VOLUME USING AIR-GAP

Carry-over is the residual solution inside the flow cell that affects the final result of a optical measurement. This quantity depends on several factors, but the most important for our purpose are:

- The density of the solution used
- The absorbance value of the solution
- The internal cleanliness of the flow cell
- The quantity of aspirated volume (sample volume)
- The flow cell volume.

The instrument carry-over must be less than 1% to pass the Quality Control. Each instrument is tested also about carry over and they are shipped only if all the Quality Control is OK.

To reduce instrument carry-over several measures can be taken:

- Use 18 µl flow cell .
- Wash flow cell to clean internally flow cell and tubes. Use 10 mL of sodium hypochlorite (6 to 9% diluted) abd WASH button, alternating air and washing solution.
- Use a greater quantity of reading solution.
- Use air-gap.

Air-gap can reduce dramatically carry-over effects, but requires a little of experience in working with it. Air-gap is an instrument features that allows the User to set up a Sample volume, a Delay time and an Air aspiration volume. Basically the instrument performs a **DOUBLE ASPIRATION** (the first for the sample , the second for the air).
In fact, after each sample the peristaltic pump is turned OFF for a programmable amount of time (**User should remove the reading solution from the aspiration spout during this delay**) and is turned ON again for a certain amount of time in order to aspirated a programmable volume of air.
Starting from fw version 1.28, an acoustic signal has been added to notify user that first aspiration has been performed, so it is possible to remove solution source to aspirate air.

This parameters are General Setup Parameters (not test parameters) so will remain the same for all the test.
The instrument is shipped with these 2 parameters set during internal QC.

Recommended value for beginner are:

DELAY = 03 s
ANSP. VOL. = 0080 uL

In this way the instrument (when a sample in flow cell is executed during a test) makes a double aspiration described in these 3 steps:

1. Aspirate the sample volume programmed for that specified test.
2. Wait for the programmed Delay time
3. Aspirate the programmed quantity of air.

You can use setup program to set this 2 parameters. Refer to General Setup paragraph in this document.

According to the test you are executing and to the test manufacturer you can change these parameters in order to fit their best values.

APPENDIX E: CUVETTE HEIGHT ADJUSTMENT

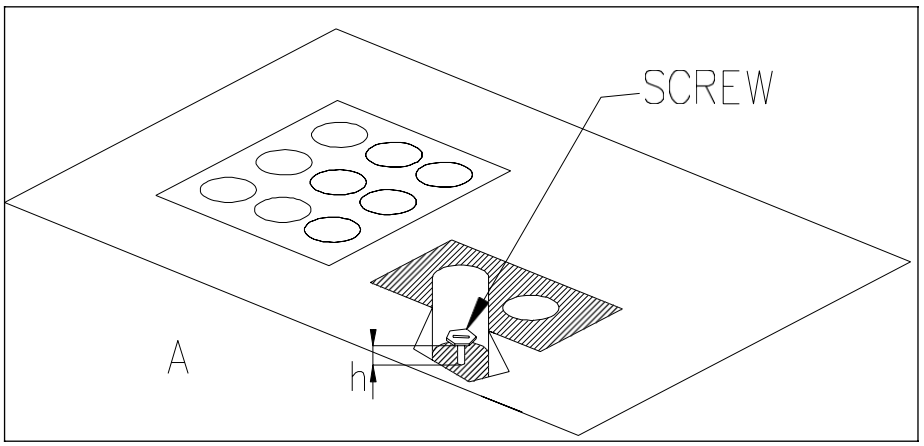
The analyzer is shipped with the reading height that exactly matches the reading aperture in the flow cell. In this condition the analyzer can work correctly in the flow cell and with a minimum reading volume of 1000 µl in standard plastic cuvette (10 mm optical path), like the ones supplied with the instrument.
Some kits however have a reading volume of less than 1000 µl (for example 800 µl). With this kit is no possible to work with the instrument unless:

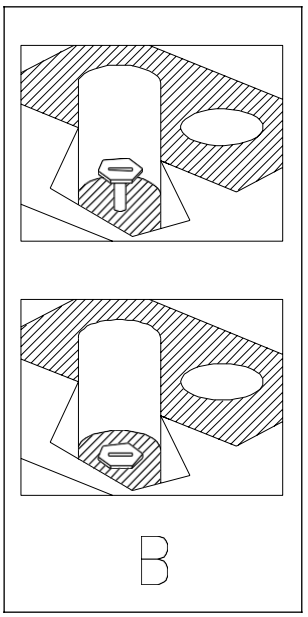
1) Use reduce volume cuvette (still 10 mm optical path). Contact your nearest distributor for more information.
2) Adjust the screw in the incubator chamber in order to increase the reading height. In this way you can reduce the volume according to your needs, but remember that the light beam does not match anymore the flow cell reading aperture and you have to setup it again if you want to work again with flow cell.

The figure below explains how to adjust the reading height (regulating the height of the screw) in order to work with flow cell or reduced volume. Refer to the following table for more information:

Screw HEIGHT (h)	FLOW CELL	REDUCE VOLUME CUVETTE	STANDARD CUVETTE
0 * -Figure A -	OK	OK	(Minimum Volume 1000 µl)
2.5 turn * -Figure B-	NO	OK	OK

*: Note that the screw height is measured according to the turn from the down position.





APPENDIX F: WEEE and ROHS DIRECTIVES

DYASET SRL complies with WEEE EC Directive (2002/96/CE) about recycling of electrical and electronic equipment waste. This EC Directive forbid to collect no more used electrical and electronic equipment waste with normal rubbish and entrust the producers the collection and the recycling of such kind of waste.
When you have to dismiss a DYASET instrument, please don't throw it with normal rubbish, but contact DYASET or the authorizeddealer.
Wasting should be performed in the country were the instrument has been sold.

The label present on each instrument certifies that DYASET complies with WEEE Directive:

DYASET also assures that no one of the materials listed by RoHS Directive (2002/95/05) is used to build and assemble its instruments.

Appendix G: important notice about biohazard

The following notes regard this label you find on the instrument:

Working with analytical instruments for in-vitro diagnostics involves the handling of human samples andcontrols, which should be considered at least potentially infectious. Therefore, every part and accessory of the instrument which may have come into contact with such samples must also be considered as potentially infectious.

Before servicing the instrument it is very important to thoroughly disinfect all possibly contaminated parts. Before the instrument is removed from the laboratory for disposal or servicing, it must be decontaminated. Decontamination should be performed by a well-trained, authorized person, observing all necessary safety precautions.
Instruments to be returned must be accompanied by a decontamination certificate completed by the responsible laboratory manager. If a decontamination certificate is not supplied, the returning laboratory
will be responsible for charges resulting from non-acceptance of the instrument by the servicing center or from any authority's intervention.

Appendix H: INFORMATION ABOUT PRINTING LAYOUT (FROM VER 1.22)

With software release 1.22 the printing format on 3000 Evolution changed in order to improve the efficency on printing, restoring some old compatibility with Screen Master 3000 and giving the instrument the possibilities to save printing paper. The following printing format are possible:

- OFF
- REP
- PRZ
- TOT

OFF printing format

Entering a test:

```
No print
```

Executing (each) sample:

```
ID: 0001   g/dL  000.0  L*
```

REP printing format

Entering a test:

No print

Executing (each) sample:

```
001 - Albumin
ID: 0001   g/dL  000.0  L*
```

PRZ printing format

Entering a test:

```
--------------------------
  3000 Evolution
--------------------------
27/08/2007 - 16:20
001 - Albumi
--------------------------
Linear limit      7.0
High limit  5.5
Low limit         3.5
--------------------------
```

Executing (each) sample:

```
  27/08/2007 - 16:20
  001 - Albumi
ID: 0001   g/dL  000.0  L*
```

TOT printing format

Entering a test:

```
--------------------------
  3000 Evolution
--------------------------
27/08/2007 - 16:20
001 - Albumi
--------------------------
Linear limit      7.0
High limit  5.5
Low limit         3.5
--------------------------
Sample asp.   1000
Rea2 volume   0
Rea1 volume   1750
Sample volume   50

…
Standard     NO
Method       EP
--------------------------
```

Executing (each) sample:

```
--------------------------
  3000 Evolution
--------------------------
27/08/2007 - 16:20
001 - Albumi
ID: 0001   g/dL  000.0  L*
--------------------------
```