Hb-Vario Analyser

Automated Hemoglobin Testing System

USER MANUAL

Revision 1.8

Date of last revision:
05/2022
UM/38/22/B

CE IVD **Erba Lachema s.r.o., Karásek 2219/1d, 621 00**

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NOTE: This equipment has been tested and found to comply with the limits for a class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in the residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does not cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Re-orient or relocate the receiving antenna.
- Increase the separation between the equipment and the receiver.
- Connect the equipment into an outlet on a circuit different from that to which the receiver is connected.
- Consult the dealer or an experience radio/TV technician for help.

The user may find the following booklet, prepared by the Federal Communications Commission, helpful: *How to identify and resolve radio/TV interference problems*. This booklet is available from the U.S. Government Printing Office, Washington, D.C. 20402, stock N° 004-000-00345-4.

Pursuant to part 15.21 of the FCC Rules, any changes or modifications to this equipment not expressly approved by Erba Lachema may cause, harmful interference and void the FCC authorization to operate this equipment.

Preface

The Erba Hb-Vario User Manual is published by Erba Lachema. Neither the whole nor any part of the information contained herein may be adapted or reproduced in any material for, except with the prior written consent of Erba Lachema.

All information, of a technical nature, and particulars of the Hb-Vario Analyser and its use are given by Erba Lachema in good faith, but may contain errors. This manual is intended only to assist the user in the use of the Hb-Vario™ Analyser and therefore Erba Lachema shall not be liable for any loss or damage whatever arising from the use or any information or particulars in, or any errors, or omission in this manual.

Users must respect the precautions and notes intended to protect them against injuries and/or instrument damage.

Misusage of the instrument and none respect of the prescribed use and the instrument maintenance procedures will void the warranty and may result in injuries.

The Hb-Vario system is initially released for testing of HbA1c. The system will eventually be used for HbA1c and HbA2/F. HbA2/F is pending release, so screens referring to A2/F in this user manual will only be available when the release will be issued.

Revisions

Revision History:

Revision	Description	Date
1.0	Initial Release	29 January 2016
1.1	Modification: new alarms	09 June 2016
1.2	Modifications: Addition of Standby status and associated behaviors; Cleaning possibility at standby and power off, new warnings, new flags and new alarms in troubleshooting messages	19 September 2016
1.3	Modifications: for Version 1.0.1: Change in Report exporting functions, Addition of LIS Chromatogram and result table transfer, Change in Weekly maintenance instructions, Changes in calibration validation criteria	06 March 2017
1.4	Modifications: for Version 1.0.2: Change in calibration run process, Change in login screen validation criteria, Changes in sample definition validation criteria, Update in LIS screen, Changes in printer header validation criteria, Updated Change Kit & Column, Change in RT criteria, Changes in Setup validation criteria, Added Web Utility.	31 May 2017
1.5	Modifications: Updated accessories list.	31 October 2017
1.6	Modifications: Upgrade config.files, clear LIS queue option, HbA2 flag limit set to 5%, Result list in filter window.	11 April 2019
1.7	Modifications: Test flag details and messages	07 August 2019
1.8	Updated pictures with RHS door open, instructions updated for shutter operations & messages in section 5.4, 6.1, 6.5.4, 8.1.2.1.2.1. Appropriate markings added in section 6.5.3. Removed manual revision number from the header of each page. Added note in Section 8.1.2.1, 8.1.2.1.2, 8.1.2.1.4. Annual maintenance section 8.6 updated. Diagnostic chart 9.1 updated.	20 November 2019

Icons

The following icons are used on the instrument to aid the user:

	Attention:	Read the installation document
	Information:	Read the instructions carefully before attempting practically.
	Biological Risk:	Be aware that this product poses some biological risk due to the nature of the material it analyses. Take appropriate pre-cautions noted in this User Manual below.
	Storage Conditions:	Store this instrument at temperatures between 1°C and 50 °C
	IVD:	This instrument is covered by the European In Vitro Diagnostics Directive.
	CE Mark:	This product is CE marked.
	Manufacturer:	This product is manufactured for ERBA Lachema by the listed manufacturer.
	Serial Number:	Denotes the product serial number.
	Disposal:	The symbol on the product indicates that this product may not be treated as a household waste. Instead it shall be handled to the applicable collection point for the recycling of electrical and electronic equipment. By ensuring this product is disposed of correctly, you will help prevent potential negative consequences for the environment and human health, which could otherwise be caused by inappropriate waste handling of this product. Please contact your local city office or your distributor of this product. Pursuant to the EU directive 2002/96/EC

- for medical devices sold from that time by Erba Lachema the corresponding costs are divided up as described below:
  1. The concerned device delivery to Erba Lachema will be paid by the CUSTOMER
  2. Device dismantling sorting of parts and elimination of wastes will be supported by Erba Lachema according to the existing local regulation
  3. In case of a sale to a third-party the first CUSTOMER shall inform Erba Lachema of the name and address of the new owner to guarantee the device traceability and to allow it's further elimination, and shall inform of the new owner that he will pay for the delivery of the device to Erba Lachema for its elimination
  4. Otherwise the first CUSTOMER will pay all of the costs and all penalties that the legal authorities should impose on the manufacturer for default of the device elimination traceability as requested by the regulation
- For medical devices sold before that time, except in particular cases, the elimination of the device will be supported by the CUSTOMER. Upon request Erba Lachema could provide this elimination. Contact us for quotation
- For medical devices sold and used in other countries, the CUSTOMER should contact the Erba Lachema REPRESENTATIVE to be informed of his responsibilities

The following iconography is used throughout this manual to help the user:

	Warning:	Please pay special attention to notices with this mark. There is potential for risk to the operator or instrument safety. There are 3 levels of warning messages: 1. Warning messages Black on White displays important user information to give elements to make informed decisions 2. Warning messages Black on Orange displays important information that can lead to some degree of system inconvenience (like damage to columns that can be easily fixed by replacing the consumable) 3. Warning messages White on Red displays important massages that can lead to damages to the instrument or potential danger to the user
	Note:	This point is worthy of note.
	Information:	Read the instructions or kit insert sheets carefully before attempting practically.
	Biohazard:	Decontaminate all parts of the instrument before service intervention. Observe appropriate precautions when using this instrument, handling sample material or clinical waste; laboratory coat, gloves, protective eye wear. Consider all human-source materials, like controls and calibrators, as potentially infectious. Dispose of all liquid waste in accordance with local and national regulations. Liquid waste pre-treatment is recommended.
	Storage:	Store the instrument between 1°C and 50°C. Pay attention to other recommended storage temperatures on associated product literature.

1 Contents

2 Overview

Warning: if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired

2.1. General Description

The Hb-Vario™ Analyser is a cation-exchange HPLC instrument specifically designed to separate fractions of hemoglobin based on their net molecular charge. The Hb-Vario has a specific use in monitoring the HbA_{1c} fraction; the portion of adult hemoglobin (HbA_0) which has been glycated through non-enzymatic modification. The Hb-Vario™ will analyze both whole blood and pre-diluted samples from different sized sample receptacles mounted on a 10 position sample rack.

2.2. Intended Use

The Hb-Vario kit is intended for the *in-vitro* quantitative determination of glycated human hemoglobin, referred to as Hemoglobin A_{1c} or $HbA_{1c.}$ (IFCC mmol/mol and NGSP %) in human blood

using ion-exchange high performance liquid chromatography (HPLC) on the Hb-Vario™ automated hemoglobin testing system.

The Hb-Vario™ can also isolate and identify the most common human hemoglobin variants.

Hemoglobin A_{1c}measurementsare used as an aid in diagnosis of diabetes mellitus, as an aid to identify patients who may be at risk for developing diabetes mellitus, and for the monitoring of long-term blood glucose control in individuals with diabetes mellitus.

The Hb-Vario kit and Hb-Vario™ automated hemoglobin testing system are intended for Professional Use Only.

Calibrators:
Hb-Vario HbA1c Calibrator Set CM is for the calibration of the Hb-Vario™ automated hemoglobin testing system for the quantitative determination of as Hemoglobin A_{1c}in human whole blood.

Controls:
Hb-Vario HbA1c Control Set is for the estimation of test precision and the detection of systematic analytical deviations of Hemoglobin A_{1c}assay on the Hb-Vario™ automated hemoglobin testing system.

2.3. Summary and Explanation

Human hemoglobin, inside erythrocytes, undergoes a non-enzymatic reaction with glucose and other saccharides derived from it. The rate and extent of this reaction is dependent on the average blood glucose throughout the lifetime of the erythrocyte.

There are several reaction products collectively known as Glycohemoglobins. The most abundant of these is HbA_{1c}. The ratio of HbA_{1c} to the total HbA concentration has proved to be a reliable indicator of the degree of metabolic control in diabetic patients.

IFCC HbA1c (mmol/mol) (mmol HbA1c/mol Hb_{TotA})	NGSP HbA_{1c} (%)	eAG (mg/dL)	eAG (mmol/L)
31	5	97	5.4
42	6	126	7
53	7	154	8.6
64	8	183	10.2
75	9	212	11.8
86	10	240	13.4
97	11	269	14.9
108	12	298	16.5

Referenced from the NGSP website (15 DEC 2014)

2.4. Scientific Principle

The Hb-Vario™ uses cation-exchange High Performance Liquid Chromatography (HPLC) to separate hemoglobin fractions in lysed human whole blood samples.

Once aspirated by the system, whole blood is diluted with a hemolyzing solution in order to break open (lyse) the erythrocytes. The lysed sample is then incubated, to remove particular interfering compounds, and then injected into a high pressure reagent stream.

This reagent stream (known as a mobile phase once it is moving through the column) carries the liberated hemoglobin into a column of tightly packed negatively charged particles. The positive charges on the surface of the hemoglobin molecules bind to the negatively charged particles in the column, preventing the hemoglobin in the sample from exiting the column with the moving reagent stream.

A second reagent is then progressively mixed into the first reagent, whilst conserving the overall flow rate of the system. This second reagent has an increased ionic strength and these ions begin to compete with the hemoglobin for the negatively charged binding sites on the column. In addition, the pH of this second reagent is different from that of the first, offering optimized separations of otherwise difficult to differentiate fractions. Different hemoglobin fractions have different numbers of positive charges on their external surfaces and therefore, different binding strengths (affinities). The controlled increase in the ionic strength of the mobile phase is used to displace the different hemoglobin fractions in a controlled manner, ensuring they exit the column at different times and with optimized separation.

The separated fractions, washed from the column, are measured using a UV-VIS absorbance detector set to 415 nm ± 5nm. The magnitude of the detector signal is proportional to the concentration of hemoglobin moving through the detector cell at any point in time. Since the hemoglobin fractions have been separated by the system prior to reaching the detector cell, higher intensity signal at any time indicates a higher concentration of a particular hemoglobin fraction. The detector signal is monitored in real time over the entire assay period and then subsequently analyzed by a computer algorithm.

2.5. Specifications

2.5.1. General Specifications

Instrument Dimensions (mm) :	369 (W) x 565 (D) x 444 (H)
Instrument Weight :	23 Kg
Operating Environment	
• Altitude:	< 2000m
• Temperature:	17-32°C
• Humidity:	10-90% (non-condensing)
Storage Environment	
• Temperature:	1-50°C
• Humidity:	5- 90% (non-condensing)
Analyzer Type:	Ion-exchange HPLC

2.5.2. Power Specifications

Power Input Requirements:	100-240 VAC 50-60Hz 250VA
Power Consumption:	150W
Over Voltage Protection:	Yes
Maximum input voltage range:	90-264VAC
Protection class	1
Pollution degree	2

2.5.3. Controlling PC Specifications

Operating System:	Windows Embedded Standard 7
Storage Medium:	32 GB SSD
Working Memory:	2 GB RAM
LIS Interface Type:	RS232
Data Export Methods:	USB / LIS / Print / LAN

2.5.4. User Interface Specifications

Display Type:	Resistive Color Touch Screen
Display Resolution:	800 x 600
Active Display Area Dimensions (mm) :	170.4 (H) x 127.8 (V)

2.5.5. Sampling System Specifications

Sampler Handler:	10 Position Rack Loader
Tube Types Accepted:	Uncapped 13 x 75 mm Uncapped 13 x 100 mm Uncapped Microcapillary Tubes (Microvette® Sartstedt) Uncapped Microcapillary Tubes (other brands than Sarstedt) defined as sample vials Uncapped 1.5 ml Sample Vials

Instrument Rack Capacity:	1 Rack
Sampling System:	Sample Aspiration Probe coupled with a high precision, maintenance free syringe of 1000µl
Sample Aspiration Volume:	*14.3µl in Whole blood, 670µl in lysate*
Sample Dilution:	*1/47*
Sample type	Whole blood on EDTA K3 or K2 anticoagulant Lysate from EDTA whole blood samples

2.5.6. High Pressure Pump Specifications

Pump Type:	Two Syringe Pumps
Priming System:	Automatic Priming & Purging
Flow Rate Range:	Minimum: 0.04 ml/min Maximum: 2 ml/min
Maximum Pressure Rating:	30 bar (435 psi)

2.5.7. Injection Valve Specifications

Injection Valve Type:	6 Port Rotary Valve with Sample Loop
Loop Size:	10 µl

2.5.8. Sample Incubator Specifications

Incubator Type:	High Temperature Incubator for the physical removal of Labile HbA_{1c}
Temperature Target:	60°C
Temperature Accuracy:	0.2°C
Temperature Stability:	0.3°C

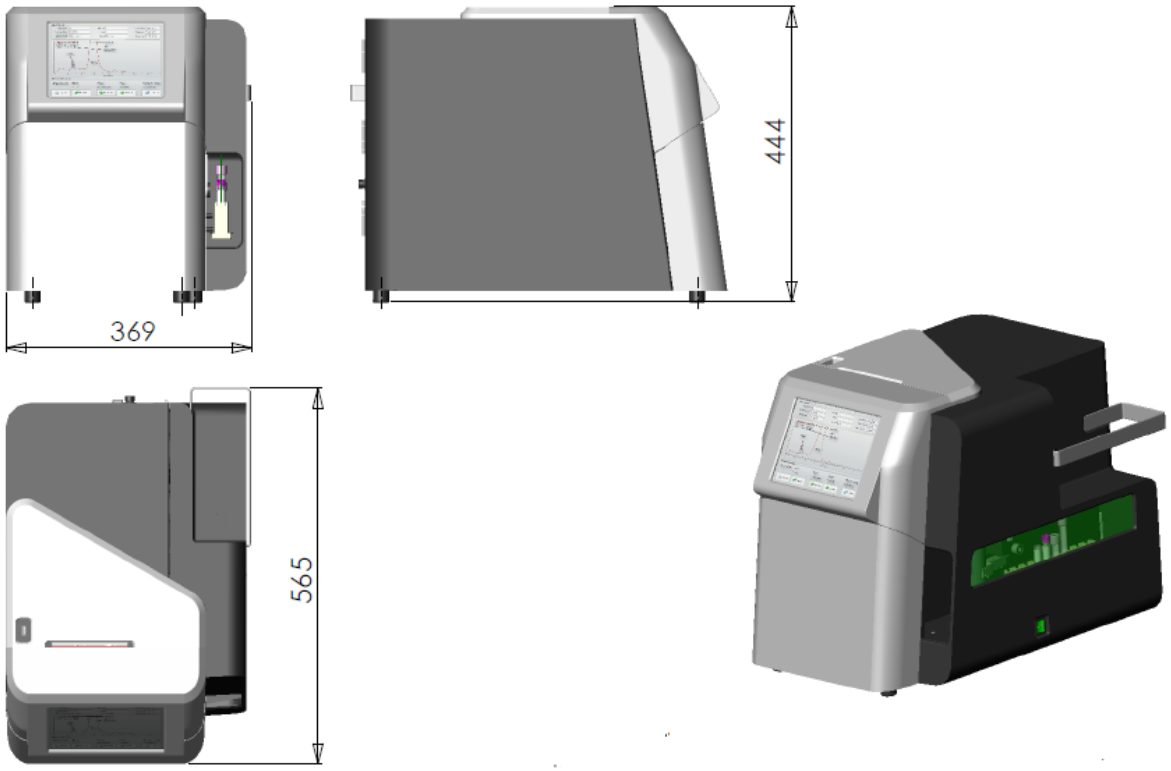
2.5.9. Column holder Specifications

Column Type:	Erba Lachema Hb-Vario Column
Temperature Target:	24.5 to 26°C
Temperature Accuracy:	0.1°C
Temperature Stability:	0.2°C

2.5.10. Detector Specifications

Detector Type:	UV/Vis
Wavelength:	415 nm
Flow Cell Volume:	80µl (estimated)
Flow Cell Path Length:	11.6mm
Linearity:	> 3 O.D.
Baseline Noise:	< 0.001 0.D.
Baseline Drift:	< 0.001 O.D. per cycle

2.6. Hb-Vario overall drawing

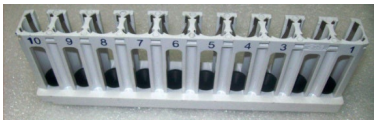


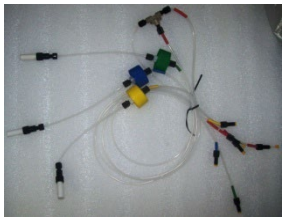
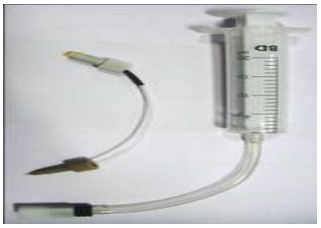
2.7. Hb-Vario identification label

The instrument is labeled according to regulations and norms. The label includes symbols that are explained in the Icons section of this manual.

2.8. Hb-Vario accessories list

The Hb-Vario analyser is delivered with the following accessories:

SR. NO.	PRODUCT CODE	DESCRIPTION	QTY.	REMARKS
1	--	Operator Manual CD	1 No.	Also available from the instrument software help with the ? button
2	170592	Assembly sample rack (DS55+ 12-00)	2 Nos.	
3	171134	Assembly high magnetic adaptor for “microvette” microcapillary	10 Nos.	
4	171135	Assembly low magnetic adaptor for sample vial	10 Nos.	
5	170287	External Universal supply AC 90-240V input, DC 24V output, 150W (DT150PWPW240C)	1 No.	
6	170551	IEC 320 C13 Power chord (adapt as per country) (626-6616 (IEC 320 C13)	1 No.* Depending on country	 
7	106046	Power cord Indian plug st 250vac/6a - ia6a3 3 X 0.75 Sq. Mm 1.8 Mtr. Length	1no.* Depending on country	 
8	170627	Microtube sample vials (220-0149)	10 Nos.	

SR. NO.	PRODUCT CODE	DESCRIPTION	QTY.	REMARKS
9	170554	Thermal paper roll 110/50/12 mm (3421272)	1 No.	
10	170365	Axis paper roller (DS5+ 05-03)	1 No.	
11	171014	2 Liter-can for Hb-Vario (Waste Can)	1 No.	
12	-	Reagent A (Blue), Reagent B (Yellow), Hemolyser (Green), Waste (Red) tubing assy. with cap.	1 Set.	
13	105067	Sample tube with barcode label	5 Nos.	
14	171122	Air removal kit	1 set	
15	101677	Cleaner for probe	1 No.	

2.9. Instrument's modules overview

1= touch screen

2= Front door (access to column)

3= Thermal printer and paper installation

4= Reagent pack lodgement

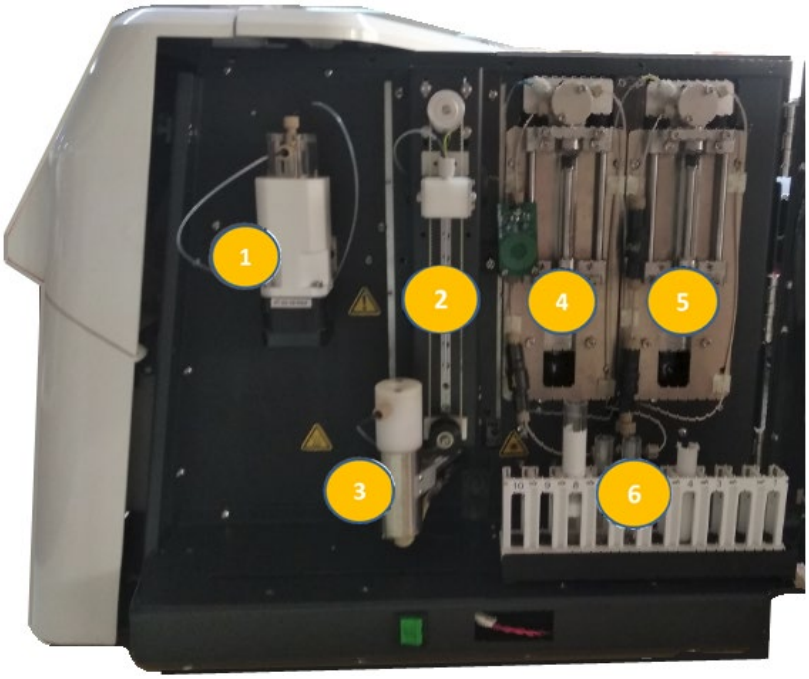
5= Sample Rack in loading/unloading position with shutter

6= ON/OFF switch

Figure 1: Closed right view

Right door opening

Figure 2: Open Right View (far)



1= Hemolyser syringe (low pressure)

2= pipette and pipette rail

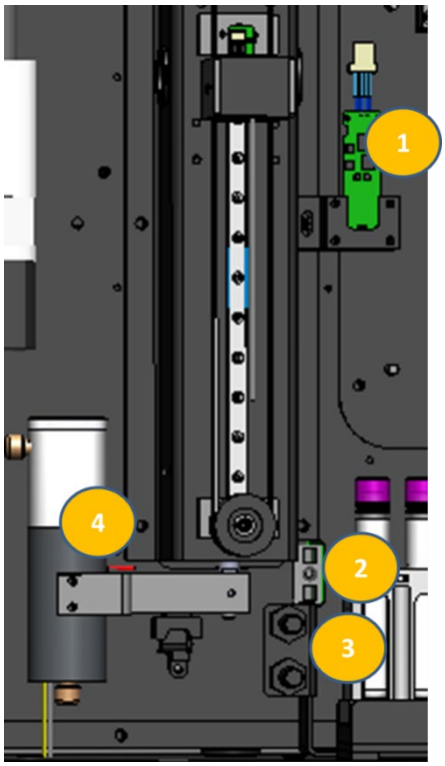
3= Incubator

4= Reagent A syringe (high pressure)

5= Reagent B syringe (high pressure)

6= sample rack

Figure 3: Open Right View (close)



1= Reagent RFID antenna

2 = Barcode reader

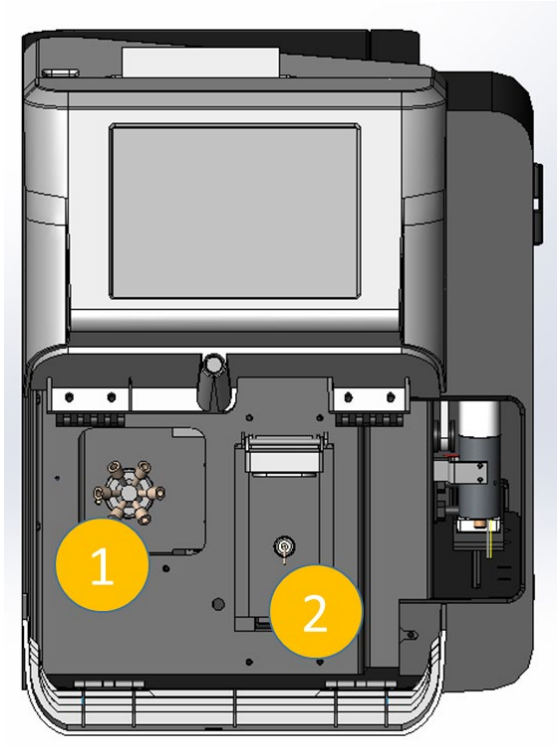
3 = Magnetic sensors (for the magnetic adaptor detection)

4= incubator in home position (sample rack moving, sample aspiration)

Figure 4: Details of right view (close up)

Incubator in dispensing and incubation position

Figure 5: Incubator view



1= Rotary valve with Loop

2 = Column holder assembly

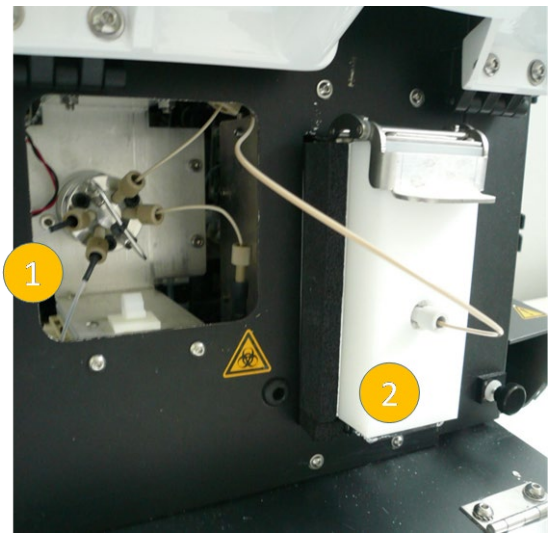
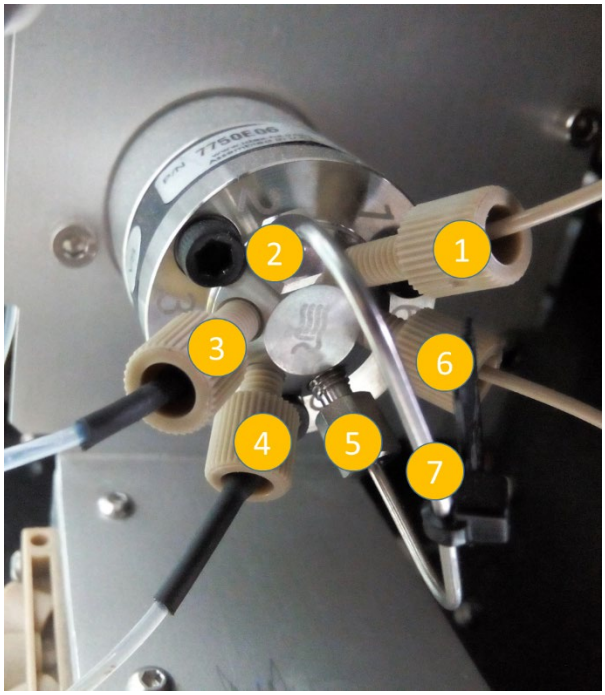


Figure 6: Open front panel



1= INPUT HIGH PRESSURE connector n°1

2 = LOOP TOP CONNECTION connector n°2

3= INPUT LOW PRESSURE (lysate injection) connector n°3

4= OUTPUT LOW PRESSURE connector n°4

5= LOOP BOTTOM CONNECTION connector n°5

6= OUTPUT HIGH PRESSURE connector n°6

7= LOOP

Figure 7: Close up view of the rotary valve and loop

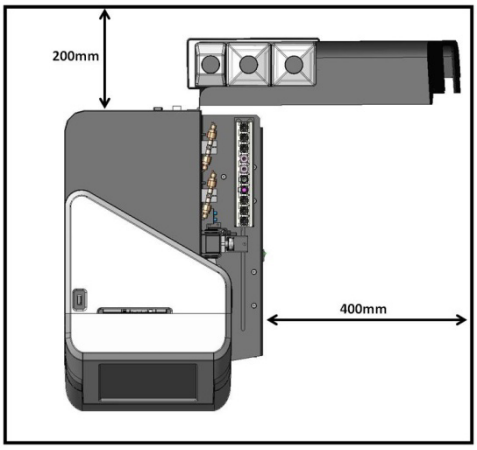
Figure 8: Close up views of the open column holder (with and without column)

3 Installation

3.1. Site Preparation

Ensure the bench space chosen for the Hb-Vario is:

- Away from direct sunlight
- Clean and clear of obstructions
- Is a level surface
- Is a at least 369 mm (W) x 565 mm (D)
- Has at least 400 mm right horizontal clearance for maintenance
- Has at least 200mm back clearance to ensure ventilation
- Can withstand a minimum weight of 25 kg (load density > 1.5KN/m^2)
- Is within 2 m of a grounded electrical power outlet



Hb-Vario installing base requirements

3.2. System Preparation

This section will guide you through unboxing and setting up the Hb-Vario, however, it is recommended you have a distributor service engineer present during this process.

3.2.1. Unboxing the Hb-Vario

Carefully remove the instrument from the box

Unboxing the Hb-Vario

1. Cut all black external ties
2. Cut all second level ties
3. Cut all adhesive film of the external box
4. Open the box
5. Remove all 4 corners blocking elements
6. Cut the internal tie
7. Remove the top block inner element
8. Remove the accessory box from one side of the instrument
9. Remove the other accessories from the other side of the instrument

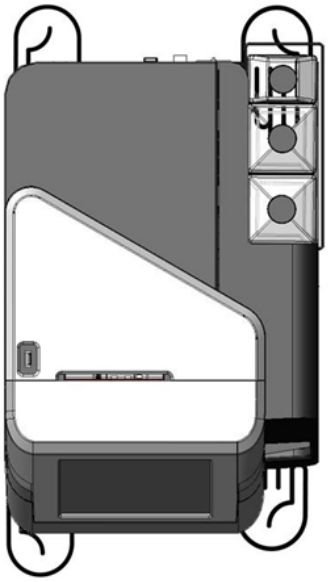
10. Lift the instrument from under (see 3.2.2 below for exact instructions on handling the instrument, this should be done by 2 persons)
11. Place the instrument on its working bench

3.2.2. Handling the Hb-Vario

Handling the instrument requires two persons

Carefully place the hands at the four legs level before carrying

Top view hands positionning

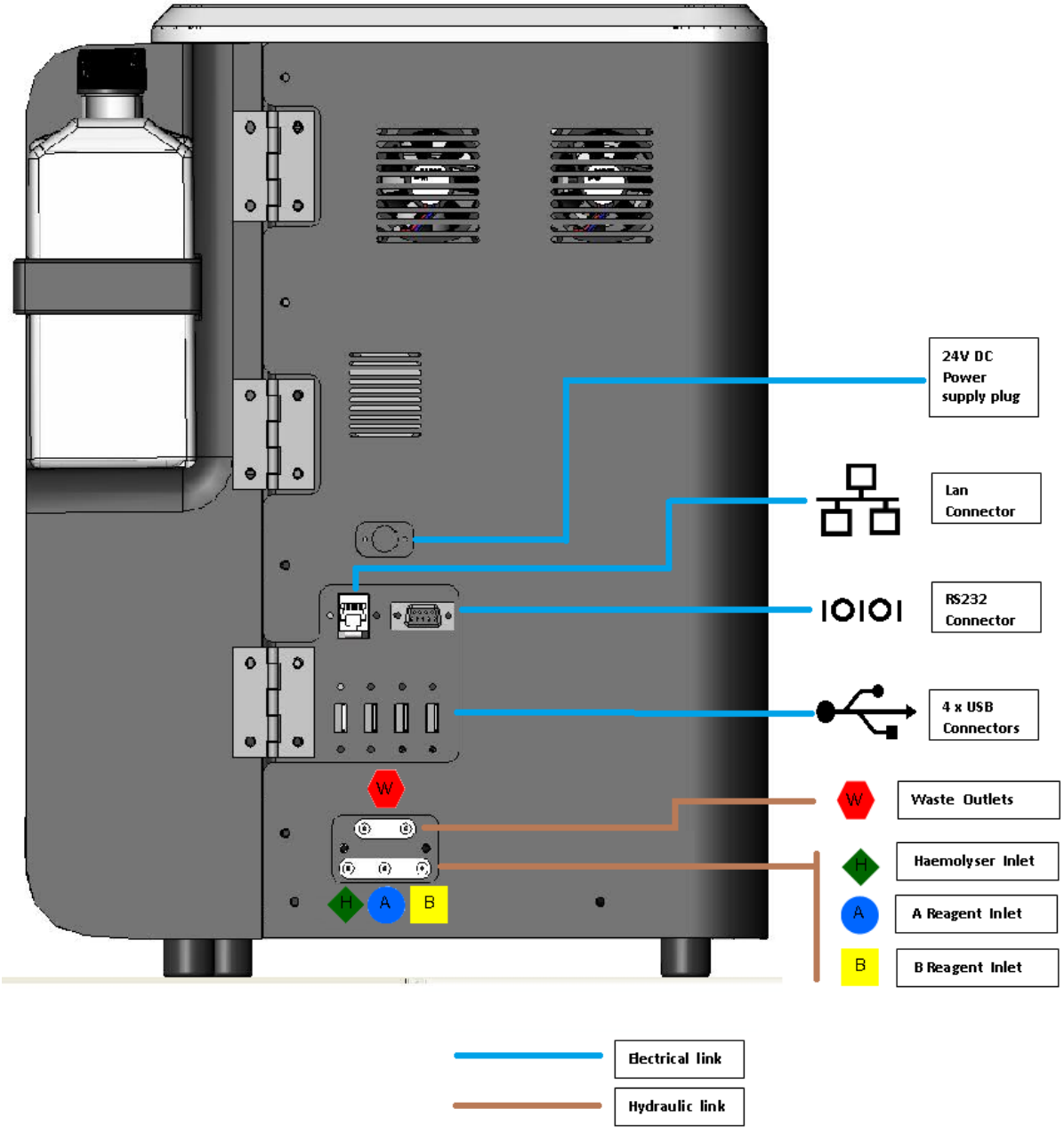


Bottom view hands positionning

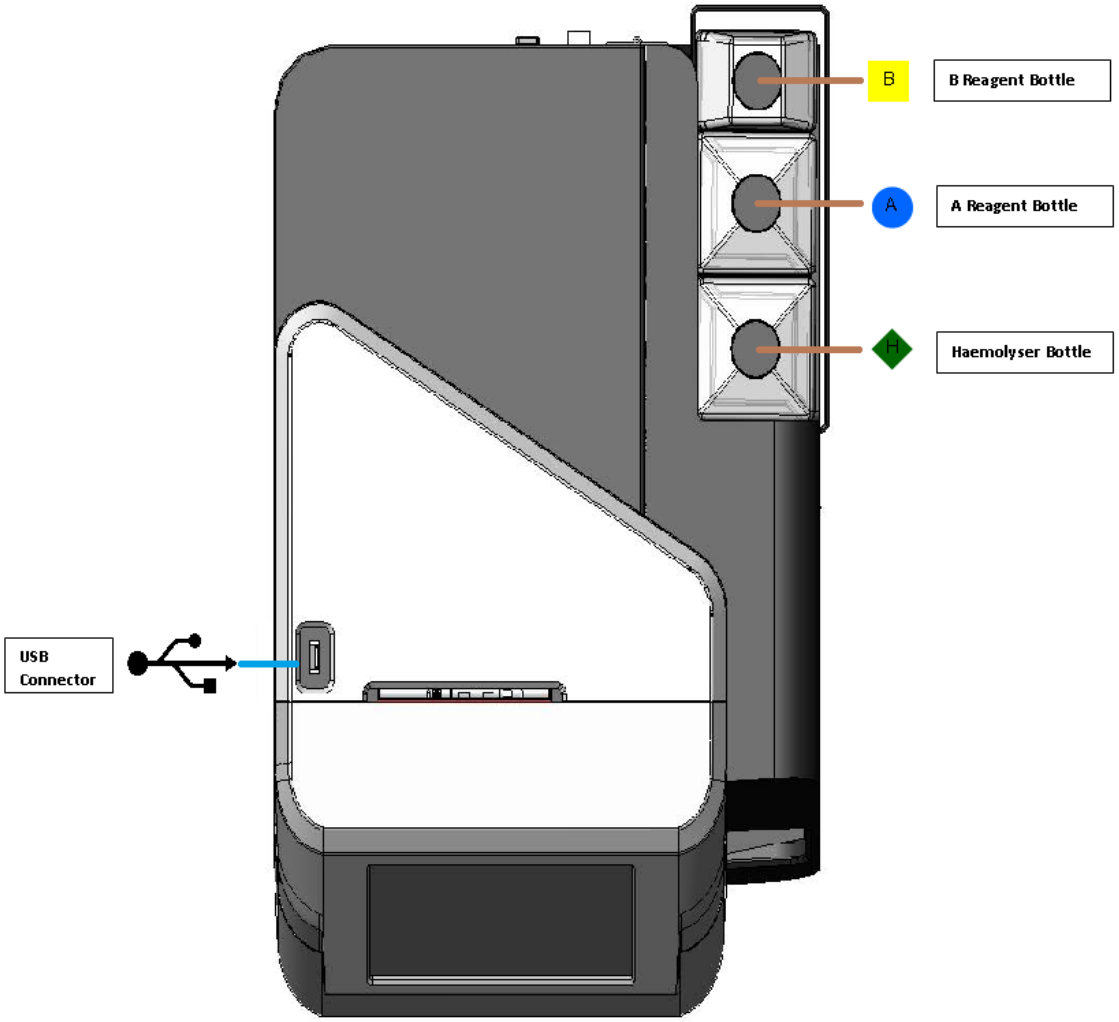
3.2.3. Unpacking the Hb-Vario

Complete the unpacking by removing all the protection material located around and inside the instrument.

3.2.4. Connection overview



Rear view


B
B Reagent Bottle
A
A Reagent Bottle
H
Haemolyser Bottle
USB
Connector

Top view

3.2.5. Precaution guide

Hardware requirements:

 Improper grounding to instrument bypasses the important safety features and may result in biased results or in permanent damage to the analyser that may void the warranty. It is necessary to ensure proper grounding. The main electrical network should comply NFC15100 standard.

 **Warning:** Installing the Hb-Vario in an area with known power supply issues such as frequent power surges or power outages is not advisable. It is recommended that the instrument be connected to an Uninterruptible Power Supply to ensure instrument safety.

 **Warning:** The safety disconnect device is the main plug. Ensure this plug remains easily accessible.

 **Warning:** For any replacement of the power cord, it must comply with IEC 320 standard and with less than 3 meters long. The minimum rated current is 5A

 **Warning:** Placing devices that can generate vibrations, such as printers, centrifuges, agitators, etc. … on the same bench as the Hb-Vario must be avoided

 **Warning:** The external USB devices should actually comply with CE mark to avoid unstable functionality

 **Warning:** Avoid dropping any liquid on surface of instrument to avoid damage

 **Warning:** Full reliability of results is only achievable with reagents and column kits provided and validated by Erba Lachema group

Bio hazard requirements:

Bio Hazard: Observe appropriate precautions when using this instrument, handling sample material or clinical waste; laboratory coat, gloves, protective eye wear.

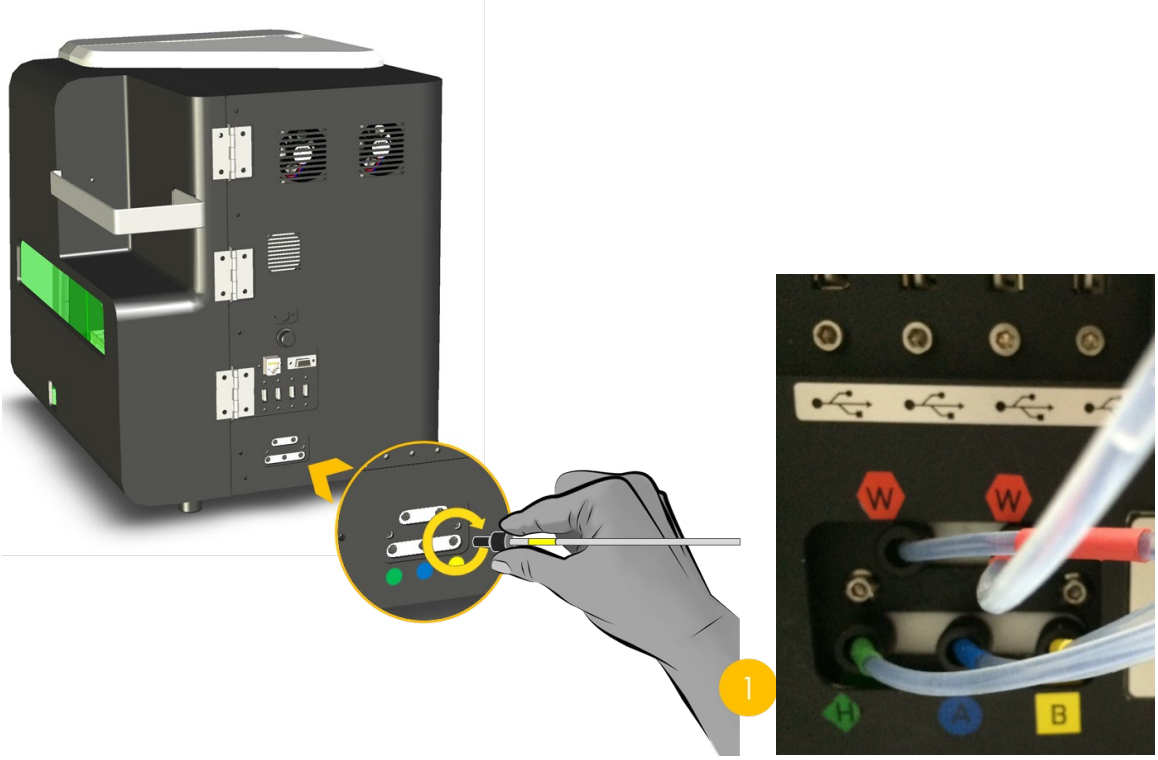
Bio Hazard: Consider all human-source materials, like controls and calibrators, as potentially infectious

Bio Hazard: Dispose of all the liquid waste in accordance with local and national regulations. Liquid waste pre-treatment is recommended

Bio Hazard: Decontaminate all parts of the instrument before service intervention

3.2.6. Install Reagent lines

Remove the Hb-Vario's reagent lines from the box of ancillary equipment provided. Install the reagent lines in the rear panel of the Hb-Vario as shown.

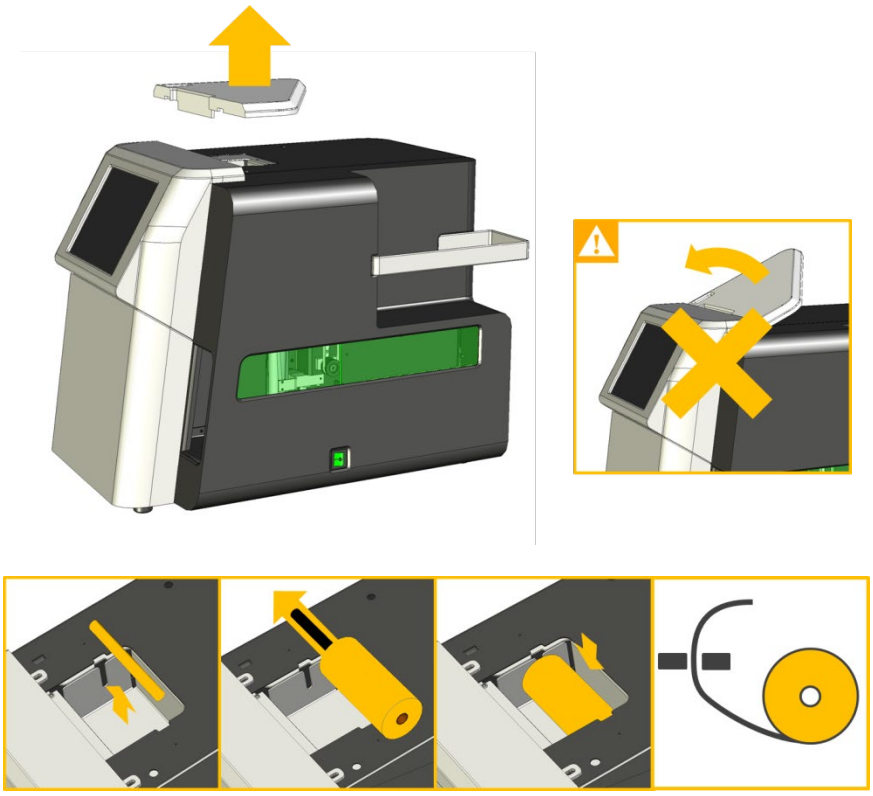


Note: the reagent lines are color coded, with shapes and or letters to avoid problems of identifications even for color blind people. **Green-Diamond-H for Hemolyser, Blue-Round-A for Reagent A and Yellow-Square-B for Reagent B**

3.2.7. Install Printer Roll

In order to fully use the Hb-Vario a printer roll should be installed so that sample, Control and Calibration reports can be printed and retained for your records.

To install a new Printer Roll, follow the steps below.



3.3. Connecting Power to the Instrument

Using the power adapter included in the accessory box, connect the Hb-Vario to a stable mains power supply.

Using the switch at the base of the right hand panel of the instrument, turn the Hb-Vario ON.

4 Start Up

Warning: The use of the Hb-Vario instrument is only intended for fully trained personnel

Once the Hb-Vario has finished booting up you will be presented with the Log In screen.

4.1. Test components

4.1.1. Required material

To run the Hb-Vario, specific reagents, columns, calibrators at minima are required. The detail of components is listed in this section. Depending on the country, these can be ordered either under a single code, or have to be ordered separately. Please consult your local representative to find out the proper way to order in your case.

4.1.1.1. Hb-Vario Kit

The Hb-Vario kit comes in 1 reference: **Hb-Vario Kit** for 200 tests, REF: **REG00038;** to run assay an additional reference is needed Hb-Vario HbA1c Calibrator Set CM, REF: **REG00039** (see 4.1.1.2)

Quantities	Items	Individual components
2	Hb-Vario Reagent Pack	Hb-Vario Reagent A
		Hb-Vario Reagent B
		Hb-Vario Hemolyser reagent
2	Hb-Vario Cleaner Solutions	Hb-Vario Cleaner
		Hb-Vario Rinse
2	Microcentrifuge Tubes	Microcentrifuge tube 1.5 ml (pack of 15)
2	“10 ml” tubes	Plastic tubes containing 10 ml for the Rinse

4.1.1.2. Hb-Vario HbA1c Calibrator Set CM

The Hb-Vario HbA1c Calibrator Set CM, REF: **REG00039** is a mandatory complement to the Hb-Vario KitREF REG00038, which contains the stationary phase and needed calibrators.

Quantities	Items	Individual components
1	Hb-Vario HbA1c Calibrator Set CM	Hb-Vario Column (2)
		Hb-Vario Level 1 Calibrator (3)
		Hb-Vario Level 2 Calibrator (3)

4.1.1.3. Hb-Vario HbA1c Control Set

As each laboratory consumes different quantities of control material depending on their workload and their quality policies, the Quality Control sets are not part of the Hb-Vario kit, but has to be ordered separately.

Hb-Vario HbA1c Control Set, REF: **REG00041**

Quantities	content	Volume of component
2	HbA1ccontrollevel 1 (0.5 ml)	0.5 ml
2	HbA1ccontrollevel 2 (0.5 ml)	0.5 ml

4.1.1.4. Hb-Vario Hemolyser Reagent

The Hemolyser reagent should be ordered as a separate reference for any user who wants to run samples or controls as **lysate**, even seldom.

- **Hb-Vario Hemolyser Reagent, REF REG00042,**
  - 1 x 900 ml of Hemolyser Reagent

Note: Some users will find that their volume activity on the Hb-Vario does not allow them to reach 100 tests with one reagent pack due to lack of Hemolyser reagent. A new bottle of Hb-Vario Hemolyser can be used to complete a kit; after the 100 tests are obtained out of a pack/column, the left over hemolyser can also be used for preparation of the lysate samples or controls without taking from the running pack.

Warning: The system RFID monitors the number of injections and volumes used by the system's injections, so no quantity of Hemolyser reagent should be subtracted from the pack bottles.

4.1.2. Hb-Vario Kit components which can be ordered separately

If additional quantities of the components included in the Hb-Vario kits are required, the following items can be ordered separately.

- **Hb-Vario HbA1c Calibrator Set**, REF **REG00040**,
  - 2 x 0.5 ml of Hb-Vario Level 1 Calibrator + 2 x 0.5 ml of Hb-Vario Level 2 Calibrator
- **Hb-Vario Cleaner Solutions, REF REG00043,**
  - 1 x 100 ml of Hb-Vario Cleaner+ 1 x 150 ml of Rinse
  - 1 x "10 ml" Tube (to position the Rinse solution on-board)

4.2. Log In

Pressing on either the 'User name' or 'Password' field will display the virtual / on-screen keyboard. Use this to enter your User name and Password(A maximum of 20 characters can be filled).

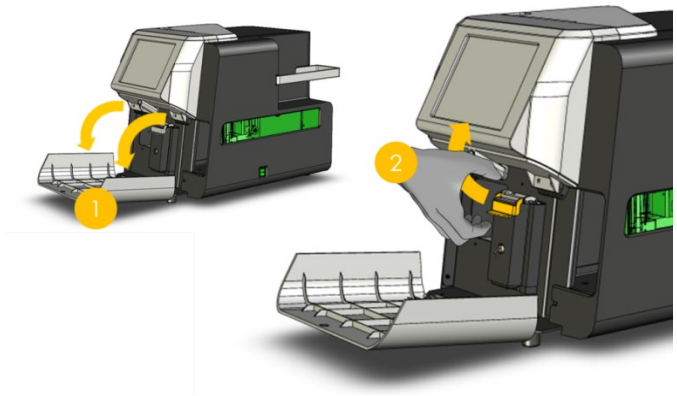
Once you have entered your User name and Password the Hb-Vario's Status Window will be displayed. The Status Window is used for controlling the instrument and its use will be covered in detail later in this manual (see sections 3, 0**,** 0, 7.6 and7.7).

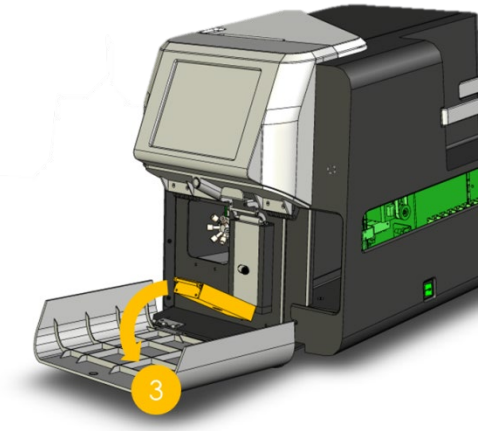
4.3. Installing the Reagents

- Open the complete kit box and take one reagent pack out.
- Take one column container from the Hb-Vario HbA1c Calibrator Set CMand place the container to one side.
- Access the reagent bottle tops.
- Remove the caps from the bottles and install the color matching instrument's reagent lines. *(when doing so, avoid shaking the tubing to avoid introducing air in the lines and touching the line filters to avoid contamination)*
- Ensure the caps on the reagent lines are secured to the reagent bottles as shown.
- Place the reagent pack in the recess provided on the right side panel of the instrument.

4.4. Installing a Column

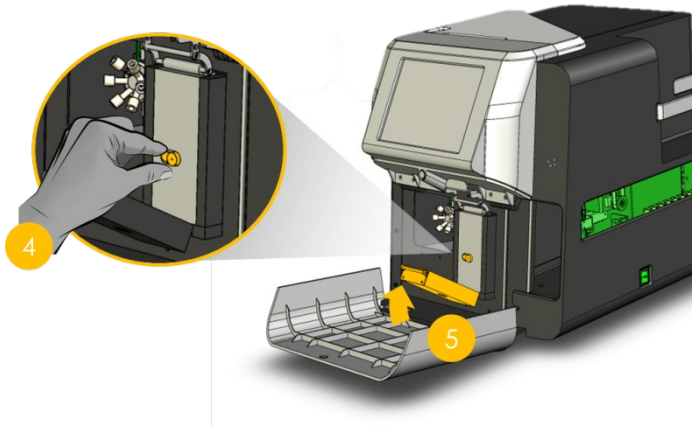
- Remove the column from the column container.
- Open the Hb-Vario's front panel and open the column holder cover.

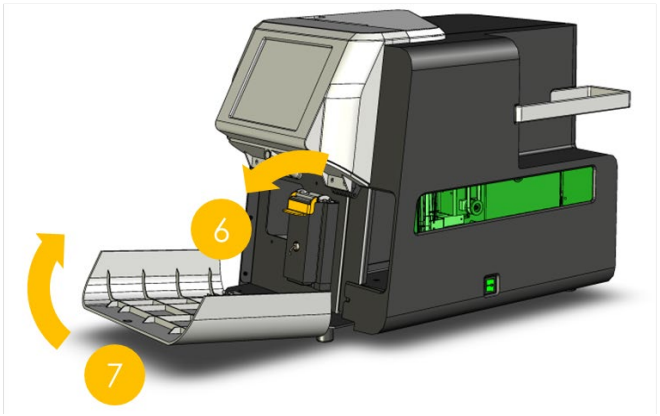




- Install the column as shown and

- Close the column holder, latching the cover closed once you have finished.

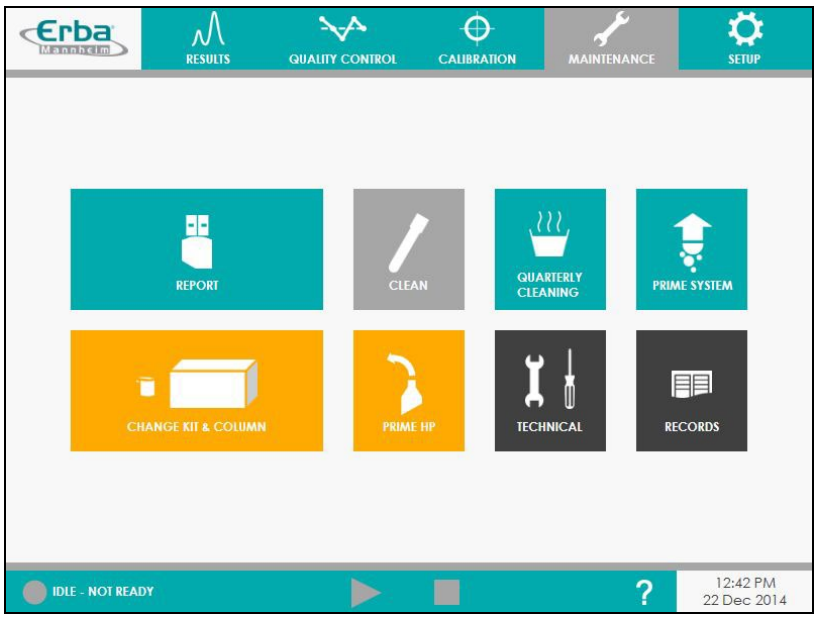




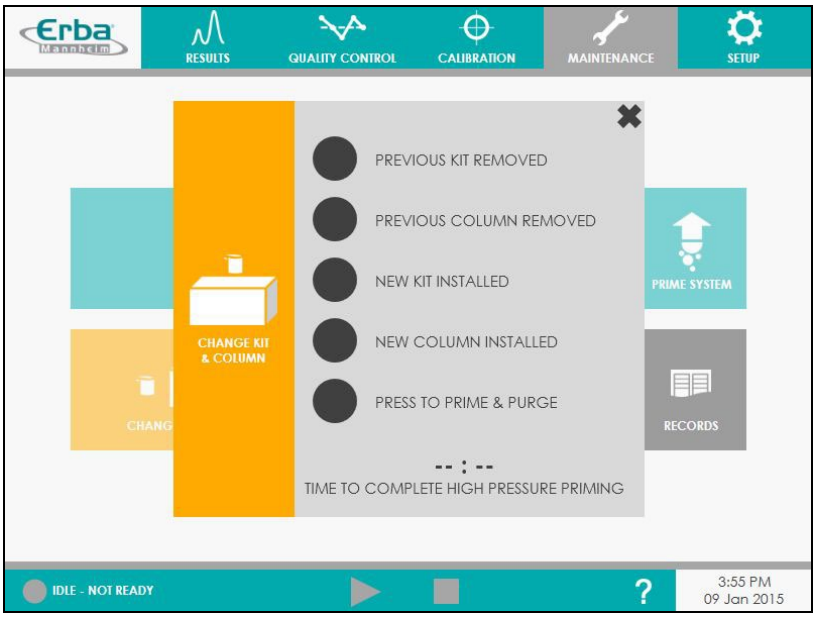
4.5. Kit Registration

Before any further work can be performed the kit which was just installed must now be registered and authenticated by the Hb-Vario. To do this, the system should be in “IDLE”, regardless of its status NOT READY, READY or STANDBY:

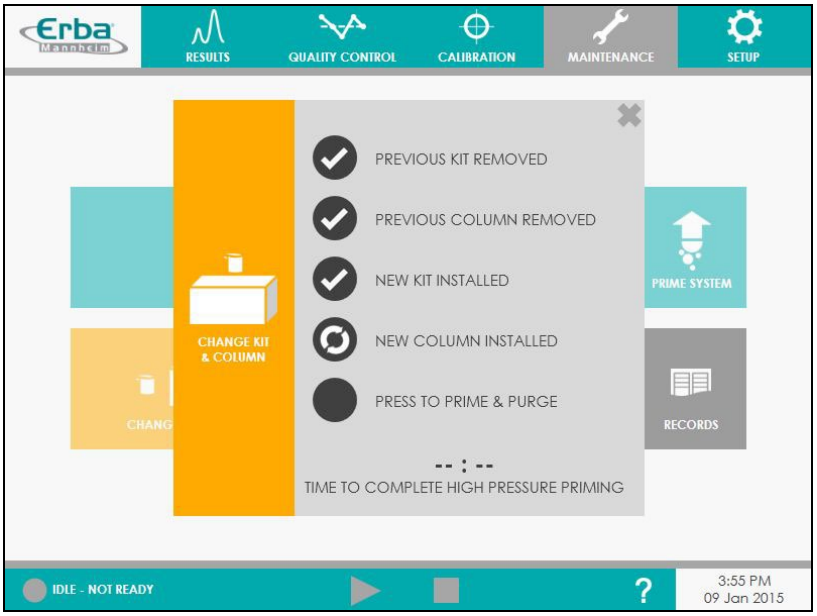
- Click on the Maintenance button from the top menu.



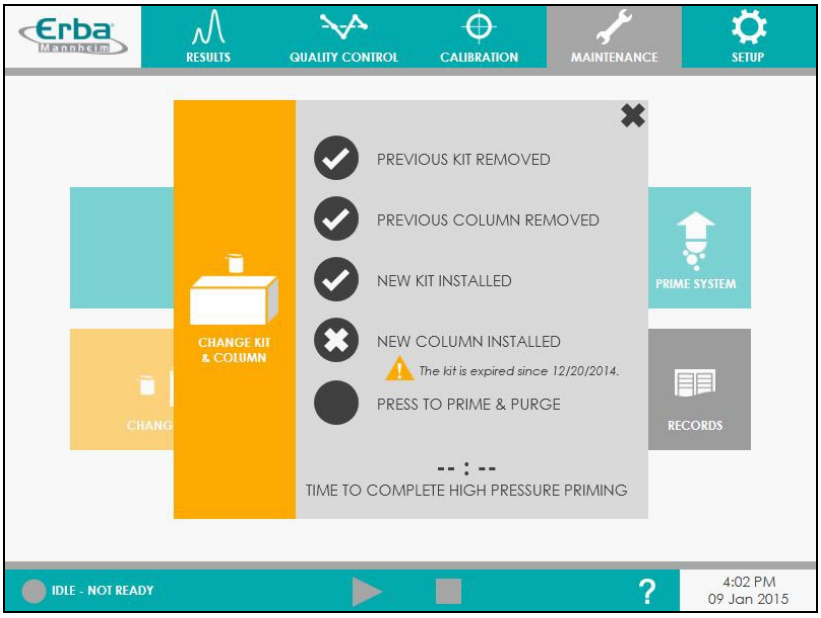
- Then press the 'Change Kit & Column' option at the bottom left of this screen. *It will open the kit installation dialogue menu; follow the on-screen instructions to complete the kit installation.*
- Click on the black dot once the step has been performed



The system will either directly display a check mark or go first to a checking procedure, which will display or revolving arrows before marking the box once the check is successful or a cross in case of failure.



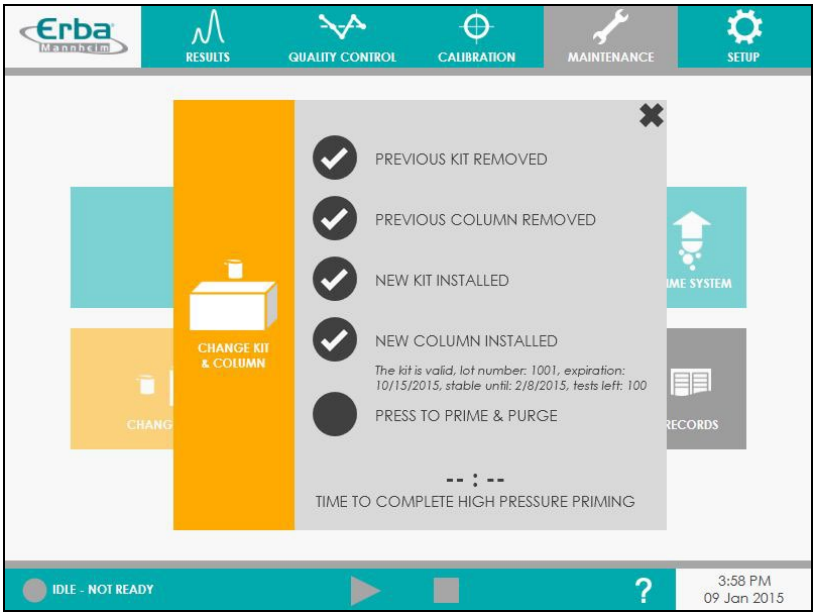
- *When you user checks that the new column is installed, the RFID tag is read; the system will authenticate the kit and column before automatically priming and purging the system.*
- If kit is not valid, the new column installed will be displayed as X



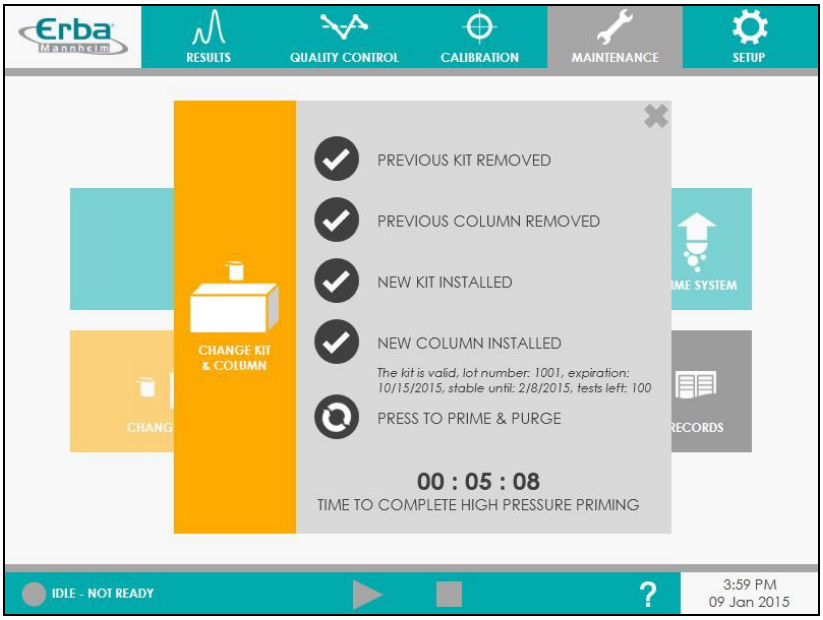
The possible messages are:

- The kit is expired since XX/XX/XXXX
- The kit is not valid for this unit.
- The kit is over, no tests available.
- The kit stability is over since X.

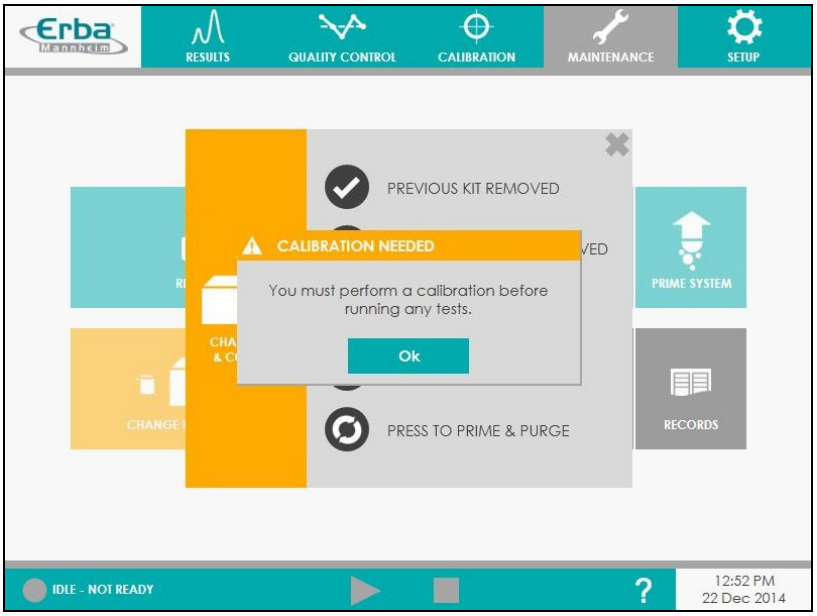
- If all ok, the following will display



- Then press prime & purge



- Just before the prime & purge is completed, a message “You must perform a calibration before running any tests” will display

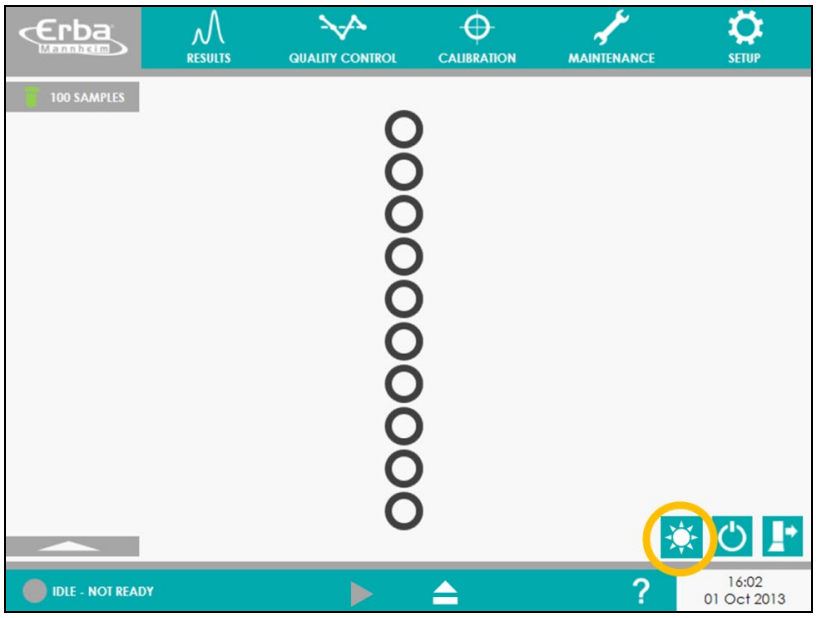


- This is just a reminder, Say OK to complete the process.

4.6. Start Up

Before any further steps can be taken, the system must run a Startup cycle. To do this:

- Press the Startup button  on the Status Window and wait until the system completes the cycle.



Once you press the Startup button the System Status Icon will change from 'IDLE, NOT READY' to 'STARTING UP, BUSY. At this point the Stop button will turn white to indicate that it is active; pressing the stop button will stop the Startup process.

Note: The stop button is also present during a Standby and can then be used to stop the Standbyin process.

Once the Startup is complete the System Status Icon will change from 'STARTING UP, BUSY to 'IDLE, READY'. In addition, the Eject button will now appear to allow the rack holder to be ejected at any time.

5 Calibration

Before reportable results can be produced by the Hb-Vario it must be correctly calibrated.

Calibration is required once per column of 100 patient/control tests. It is usually not necessary to recalibrate during the life of a 100 test column, unless the QC slowly drifts out of acceptable range. Then if fresh controls exhibit well defined chromatograms and confirm the drift, a recalibration might be needed to re-center the QC on their target values.

Read First: After securing an Hb-Vario Calibrator Kit, follow the instructions on the kit insert to prepare sufficient material for a calibration.

5.1. General information about Calibration on Hb-Vario

5.1.1. Conditioning of the Hb-Vario column

On Hb-Vario the “calibration” actually includes more than the calibration of the assay. For the initial calibration on each column, the process covers: the conditioning of the column, the auto check and possible adjustments of Retention Times and the calibration of the assay.

For ulterior calibrations, the system will again perform the auto-check of Retention Times and calibration of the assay, skipping the processes of Priming and baseline setting of the column.

This process is automated, the system knows whether the calibration is the first one for a column and programs the needed steps as the user requests the calibration.

For these reasons, during the **first calibration on a new column**, the calibrators CAN ONLY BE WHOLE BLOOD, as the calibrator Level 2 is used to “condition” or “Prime” the column.

In order to perform these primers on a new column, the number of calibrators’ injections at the time of calibration request are respectively displayed 7 and 4 or 8 and 5 depending on the calibration replicates (2 or 3) programmed in Setup;

Since the “calibration process” also auto checks and sets the Retention Times, the number of injections can evolve in real time during the process. It can go as high as 12 and 4 in duplicate and 13 and 3 in triplicate. Leading to a full duration of Priming/auto check/adjustment/calibration process going from a minimum of 47 minutes to a maximum of 1 hour 07 minutes in duplicate or from 55 minutes to 1 hour 15 minutes in triplicate.

The system will automatically aspirate 2 concentrated aspirations of Level 2 calibrator as a “primer” to condition the column, 4 blanks will be performed to obtain the baseline and 2 aspirations of Level 1 calibrator would be run to perform and auto-check the Retention times.

Blanks and repeat Level 1 will be processed until the system is correctly set for Retention Times, then the Level 2 will be processed.

Primer RT can become Level 1 Calibrator if auto-check passes on first or second check.

Subsequent calibrations on the column (if needed) can be performed as Whole blood or Lysate. Durations of Subsequent calibrations vary from 27 minutes and 30 seconds to 35 minutes and 30 seconds.

5.1.2. Running calibrators and controls as lysate

Except for the initial column calibration that requires Whole Blood calibrators, to run a sample as Lysate, samples (calibrators, controls or patient samples) are prepared with 50µl of calibrator, control or whole blood sample + 2000µl of Hemolyser to run duplicates in a 5ml tube placed directly in the sample rack, or 25µl of sample + 1000µl of Hemolyser in an 1.5ml Eppendorf placed in a sample adaptor (magnet in low position) to run a single injection.

Warning: Ensure that you select the correct sample type for controls and calibrators (Whole Blood or Lysate (Prediluted)), since running Whole Blood samples in Lysate mode may damage the column but mostly **significantly increase risk of blockage in the low pressure.**

5.1.3. Running Quality Controls to validate calibration

The only way to validate a calibration is to run Quality Control material. In order to request a calibration, the Hb-Vario requires to select Control samples that will automatically be following a calibration to ensure the system is correctly calibrated.

Warning: When calibrations and controls are programmed, the controls can be removed from the rack, and only the calibrators be run. Although this is not advised as it removes all means of checking the calibration validity, it is possible.

5.1.4. Automatic Priming and Calibration validation

Upon running a calibration procedure, the Hb-Vario system will automatically analyze and evaluate the results to assess the validity of the process.

The following checks are performed:

- Primer minimum area = 6000.
- Blank verifications:
  - Total area: ≤ 400
  - Maximum Absorbance: ≤ 50 mAbs
  - Delta area between Blank 2 and 3: ≤ 150
- For level 1 Calibrator: 6% maximum of deviation between all replicates.
- For level 2 Calibrator: 3% maximum of deviation between all replicates.
- Calibration factor between 0.97 and 1.25.
- Calibration Offset between -0.8 and 1.00
- Retention Time (RT) checks: Calibrator Level1 between 46 and 49s.
- $R^2 \geq 0.99$

If any of these fail to comply, the system will instruct the user for consecutive actions.

5.1.5. Volumes needed for calibrators and control materials

Correct volumes are :

Calibrators should be used only during the time of calibration and discarded afterwards:

- Place the full content of the reconstituted calibrator vial into a 1.5ml Eppendorf supplied in the Hb-vario kit, placed on a bottom magnet adaptor on the rack.
- The minimum volumes of calibrators needed for an initial calibration are 150µl for Level 1 and 220µl for Level 2.
- The minimum volume of calibrators needed for ulterior recalibrations is : 150µl for Level 1 and for Level 2.

Controls:

- 70µl of control material should be placed into a 1.5ml Eppendorf placed in a bottom magnet adaptor on the rack
- Or prepare a lysate with 25µl of sample+1000 µl of Hemolyser or 50µl of sample + 2000µl of Hemolyser. User can then place the lysate respectively in an Eppendorf on an adaptor (low magnet) or in a 5ml transfer tube.

5.1.6. Possibility to run samples on the calibration rack

If only **1 type of assay** (HbA1c), **6 positions** are available to run samples immediately after the calibration and control procedures.

If the 2 types of assays are to be calibrated (A1c and A2/F) then the calibrators and controls will occupy 8 positions on the rack *(respectively the HbA1c Low and High Calibrators, followed by the HbA1c Low and High Controls, followed by the HbA2/F Low and High calibrators, followed by the HbA2/F Low and High Controls).* In this case, **2 positions** are available to run samples immediately after the calibration and control procedures.

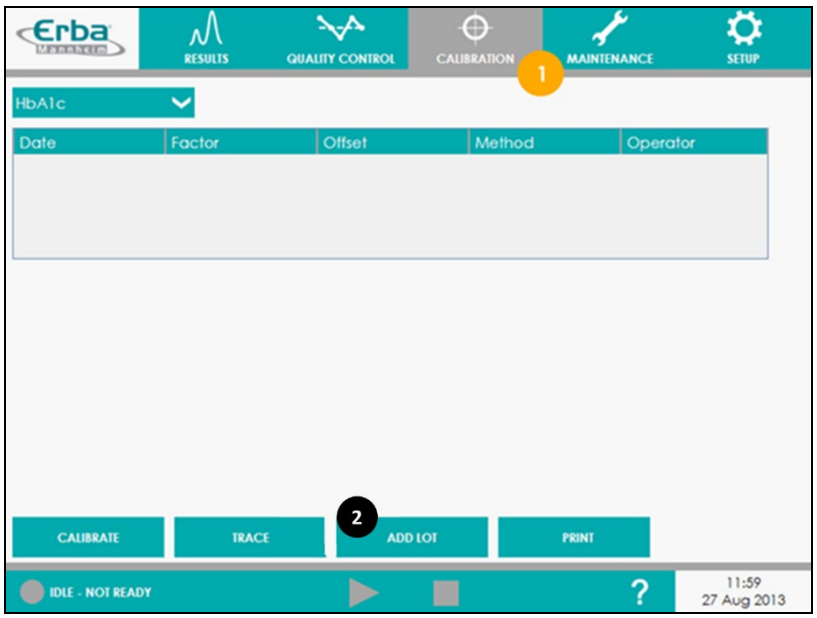
5.2. Adding Calibrator Lots

To perform a calibration:

- Press the Calibrator Window button in the Top Menu, and then select a test (HbA1c, HbF or HbA2).

- Calibrators are linked to the Hb-Vario Kit, the RFID injects the Reagents and the calibrator's lot number and values for the kit installed.
- If later, more calibrators than those provided with the kits are needed, and the ones to use are of a different lot than those already defined, then begin by adding the calibrator lot information to the Hb-Vario. To add a Calibrator Lot press the ADD LOT button.

If lot already exists, then go directly to the Calibrate button (5.3) and select the lot you intend to use. If not present, follow the steps below.

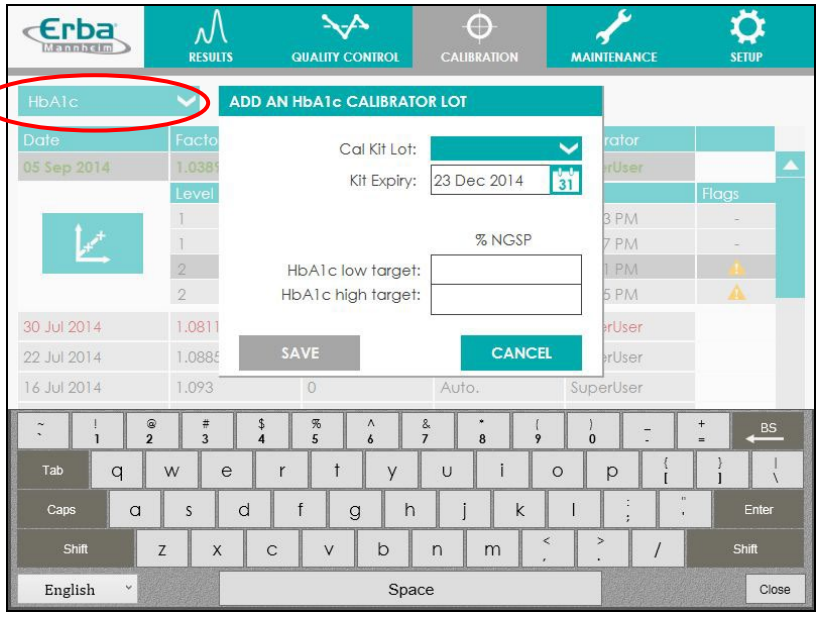


Read First: Calibrator target values can be found on the Calibrator Kit Insert Sheet.

Note: Pressing any of the text fields will bring up the on-screen keyboard.

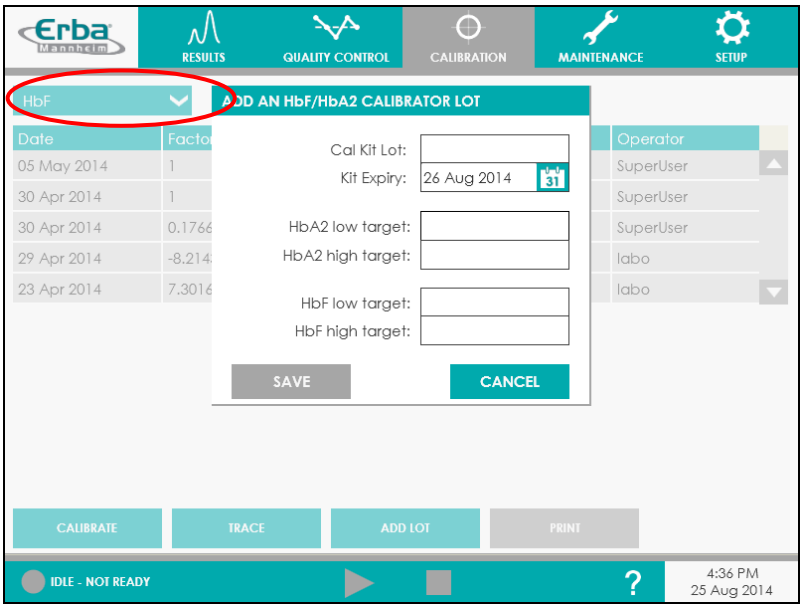
Note: Only once all of the required data has been added will the 'SAVE' button will be accessible.

For the HbA1c assay, the window is as follows:



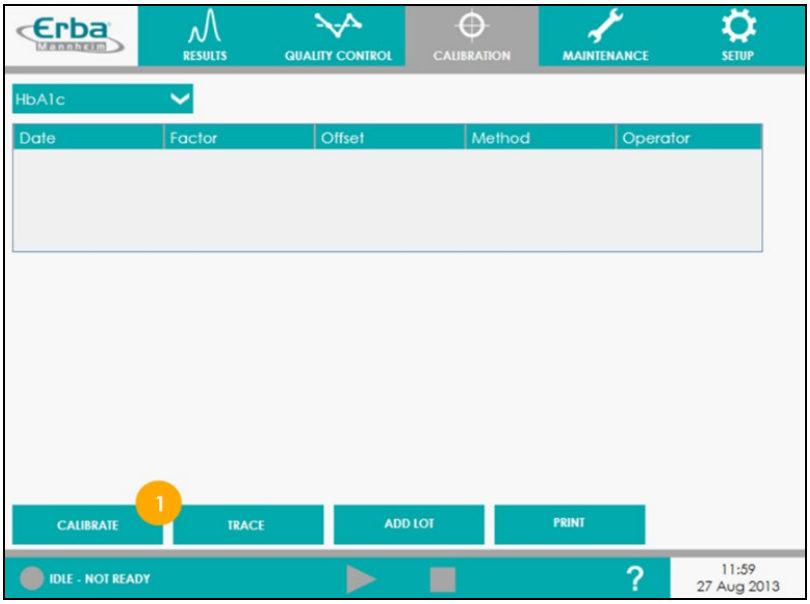
- Enter all necessary information :
  - Calibrator kit Lot (located on the kit and not on the vials)
  - The kit expiry date
  - The target values for the Low and High calibrators
- Click on the save button to save the new calibrator lot number and its related data.

For the HbA2 or HbF assays, the common window is as follows:



- Enter all necessary information :
  - Calibrator kit Lot (located on the kit and not on the vials)
  - The kit expiry date
  - The HbA2 target values for the Low and High calibrator levels
  - The HbF target values for the Low and High calibrator levels
- Click on the save button to save the new calibrator lot number and its related data.

5.3. Requesting a Calibration



Once a Calibrator Lot has been added, a calibration can be run. To start a calibration:

1. Press the **CALIBRATE** button in the Calibration Window.

2. Depending on the assay selected previously, the 'SELECT AN HbA1c CALIBRATOR LOT' (above) or the 'SELECT AN HbA2/HbF CALIBRATOR LOT' (below) dialogue opens and allows you to select a valid Calibrator lot. Press the white dropdown arrow and select the desired Calibrator Lot or the one you just added. The calibrator target values and the kit expiry fields will be filled automatically.

Note: By default, the system will select the calibrator lot that matches the kit installed. But user can select another lot if needed.

Only calibrator lots with valid expiry dates will be available from the dropdown list.

3. Select the sample type for the calibrator using the second combo box.

Note: First calibration of a column can only be run as Whole Blood.

4. Select the **Control kit lot** you are going to use during this calibration procedure in the Controls, control kit lot drop down list. The control limit values and the kit expiry fields will be filled automatically.

5. **If your lot of control is not present** in the list of valid control lots, you must first add a Control Lot. An ADD NEW LOT button conveniently accompanies the controls section in this dialogue to allow the adding of Control Lots directly - see 5.3.1 a) to c) for HbA1c or d) to f) for HbA2/F)

Warning: Ensure that you select the correct sample type for controls and calibrators (Whole Blood or Lysate (Prediluted)), since running Whole Blood samples in Lysate mode may damage the column but mostly **significantly increase risk of blockage in the low pressure.**

6. Once all of the required data has been entered, the SAVE & RUN button will become active.
7. Press the SAVE & RUN button to be transported directly to the Status Window. *The Status Window will be pre-loaded with Calibrators and Controls ready for the run, see 5.4 for running calibration*

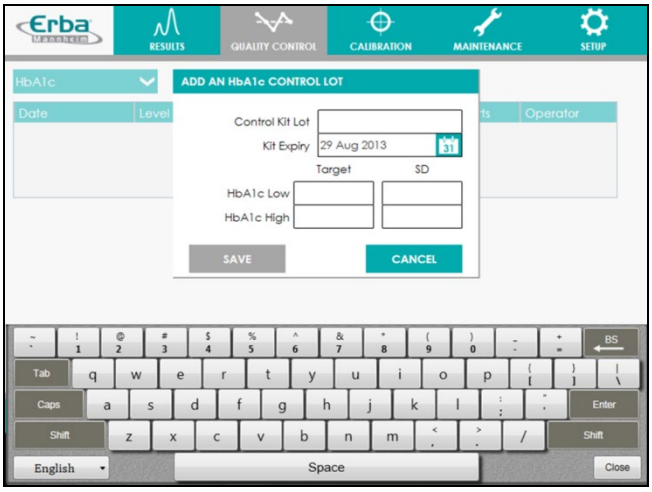
5.3.1. Add a Control Lot

To add a Control Lot you must enter both a Target (Control Mean) and a Standard Deviation for both the high and low Controls, as well as a Lot and Expiry for the Control Kit.

*This control lot creation can be done from the **Quality Control** menu (ADD NEW LOT button) or from the **Calibration**, Calibrate menu (ADD NEW LOT button from the Controls area)*

Read First: This information can be found on the Control Kit Insert Sheet along with the instructions for preparation of the material.

For the HbA1c assay, the window is as follows:



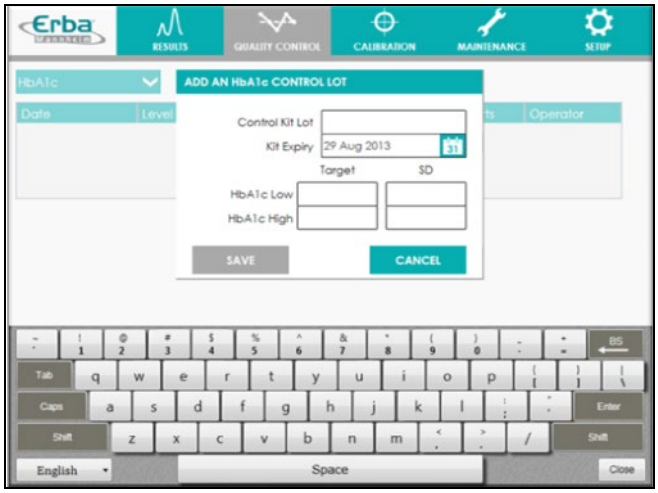
a) Enter all necessary information:

- Control kit Lot (located on the kit and not on the vials)
- The kit expiry date
- The target values for the Low and High Controls
- The SD (Standard Deviation) for the Low and High controls*(located on the control insert sheet)*

b) Click on the save button to save the new control lot number and its related data. *(the save button only becomes active once all of the required data has been entered)*

c) You will then be delivered back to the Calibration Window's Calibrate Screen, where you can select the Control lot you have added and select whether you wish to run Controls in Whole Blood or Lysate mode. (5.3, step 5)

For the HbA2 or HbF assays, the common window is as follows:



d) Enter all necessary information :

- Control kit Lot (located on the kit and not on the vials)
- The kit expiry date
- The target values of the HbA2 for the Low and High controls

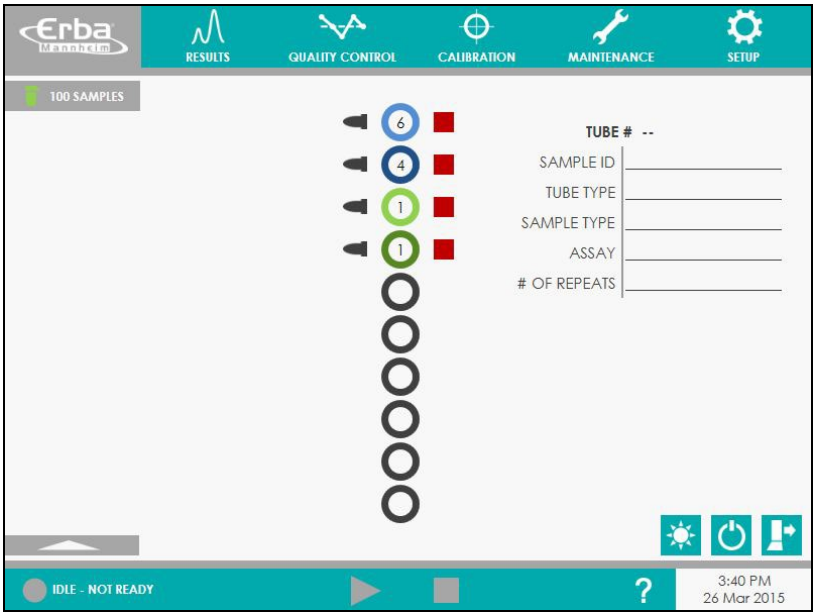
- The SD (Standard Deviation) for the HbA2 Low and High controls*(located on the control insert sheet)*
- The target values of the HbF for the Low and High controls
- The SD for the HbF Low and High controls

e) Click on the save button to save the new control lot number and its related data. *(the save button only becomes active once all of the required data has been entered)*

f) You will then be delivered back to the Calibration Window's Calibrate Screen, where you can select the Control lot you have added and select whether you wish to run Controls in Whole Blood or Lysate mode (5.3 step 5).

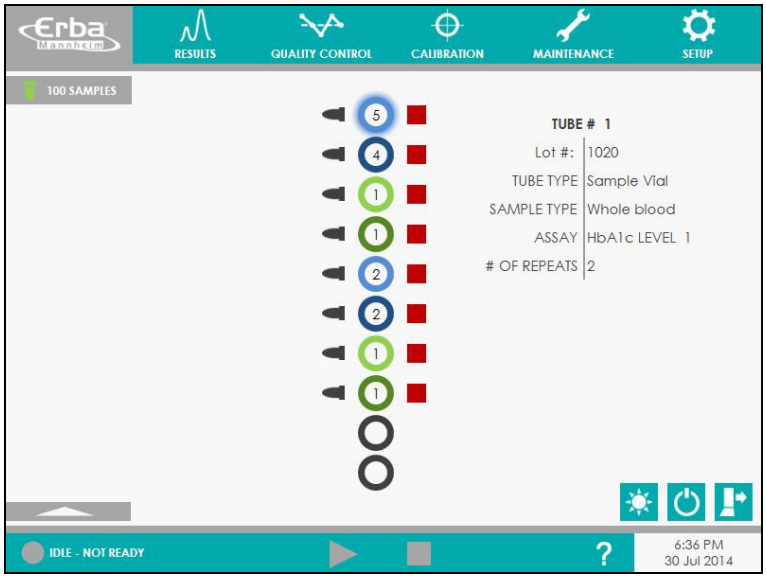
5.4. Running the Calibration

After requesting the calibration and pressing the SAVE & RUN button, the status window preloaded with calibrators and controls display as programmed



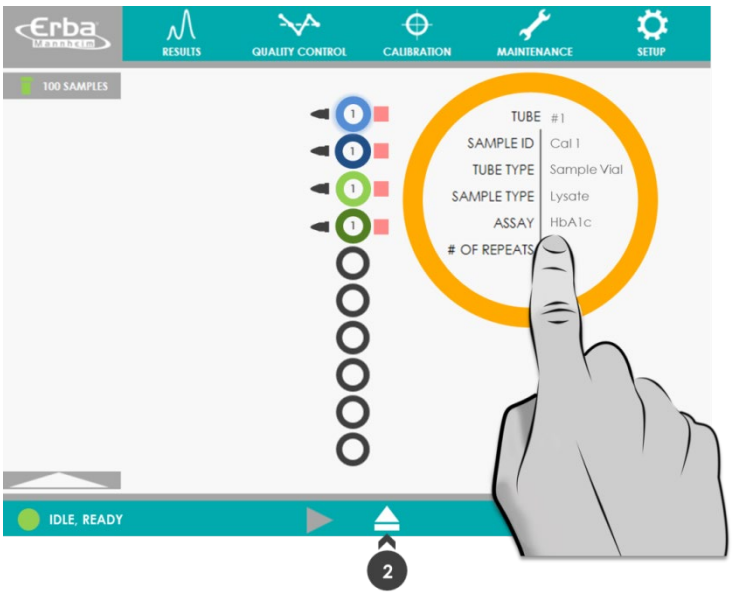
Upon requesting a calibration for only 1 type of assay, the rack loading is as displayed above. 6 positions are available to run samples immediately after the calibration and control procedures.

If the 2 types of assays are to be calibrated (A1c and A2/F) then the following rack will be programmed. *(Corresponding to the HbA1c Low and High Calibrators, followed by the HbA1c Low and High Controls, followed by the HbA2/F Low and High calibrators, followed by the HbA2/F Low and High Controls).*

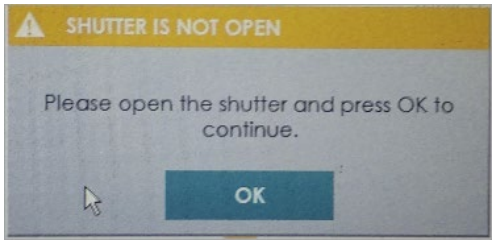


In case of calibration of the 2 assays (HbA1c and HbA2/HbF), 2 positions are available to run samples immediately after the calibration and control procedures.

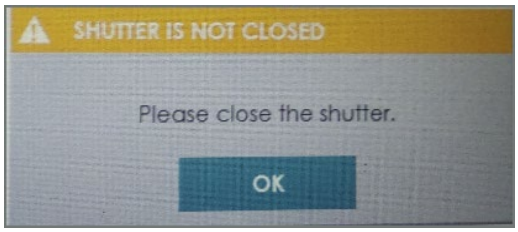
Note: The tube type can be changed prior to the start of the run. To do this, select the sample you wish to alter and then press anywhere in the in Selected Sample Data area as shown below.



- Press the eject button (highlighted above 2). To open the shutter message will get displayed to open the shutter.



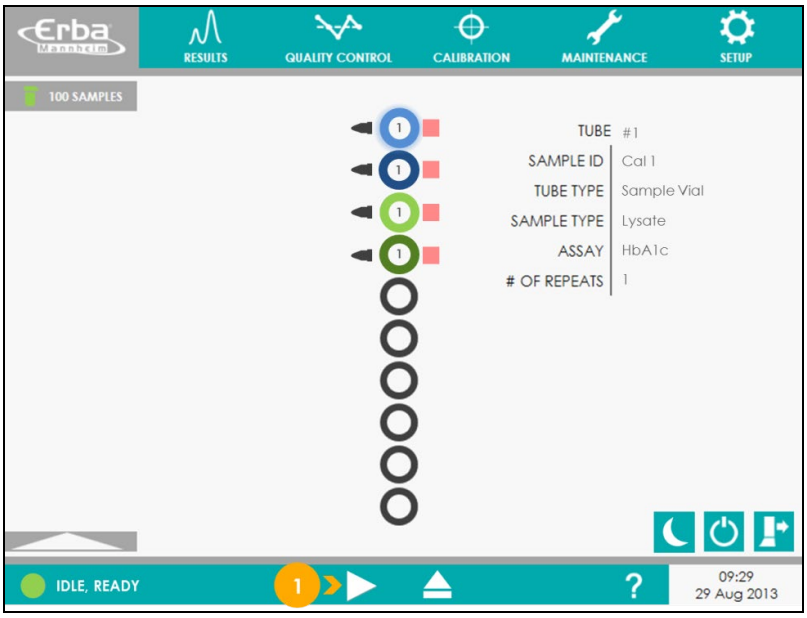
- Open the shutter and click OK , sample rack holder will move to the loading/unloading position, ready to accept the sample rack.
- After loading /Unloading sample rack, message will get displayed to close the shutter.



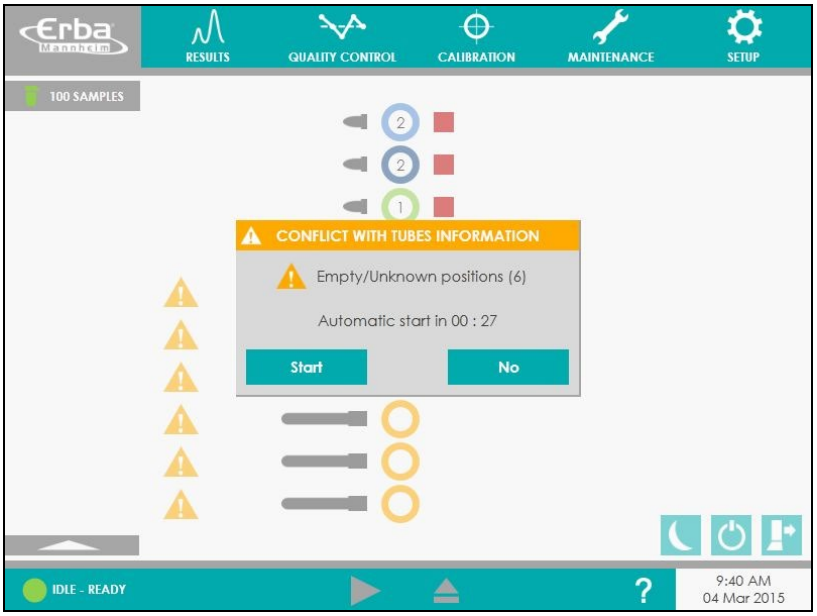
- Close the shutter and press OK to continue.

Warning: Dispense appropriate volumes of control and calibrator materials into the appropriate vials as directed by the Control Kit and Calibrator Kit inserts and the rack loading on the software. (See5.1.5)

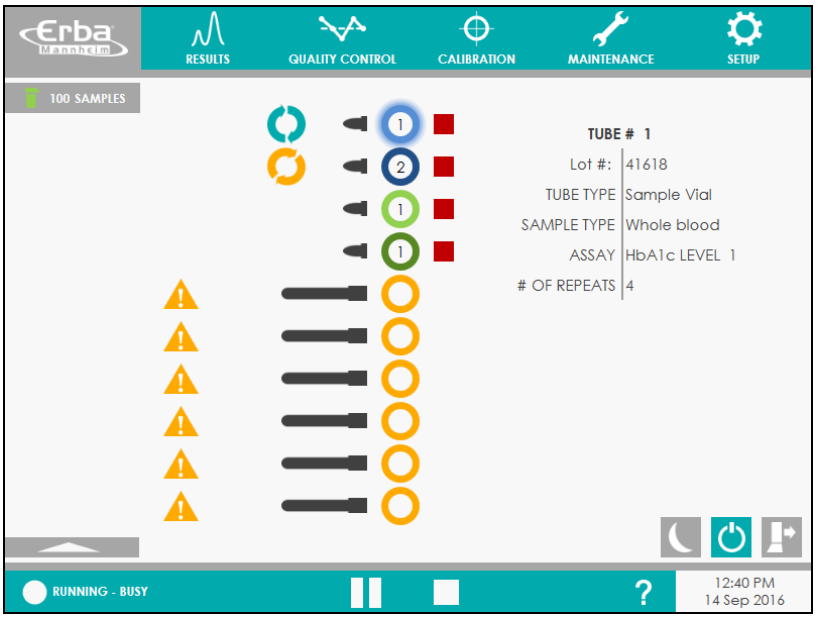
- Insert the tubes of control and calibrator materials into the sample rack in the correct positions and order. Place the sample rack into the sample rack holder and press the Run button.*(see Loading samples 6.5 for more details about tubes, adaptors and rack loading)*



When the system begins the run, if the rack is not filled the following will appear. Press Start to continue if the empty positions are intended. (For more details please see 6.5.4 Running, Loading samples, loading)



1. As soon as a sample is calculated by the system the number of samples left on the column is debited.
2. The sample being prepared will feature an orange / yellow rotating arrow. Any sample being analyzed features a blue / teal rotating arrow.
3. The System Status Icon change color and the system status changes to 'RUNNING, BUSY'.



Note:

A sample is being prepared if it is:

a) Being aspirated from the sample vial / tube

b) Being incubated

c) Being injected onto the Column

A sample is being analyzed if it is:

a) Injected onto the high pressure column and measured

5.5. Reviewing the Calibration

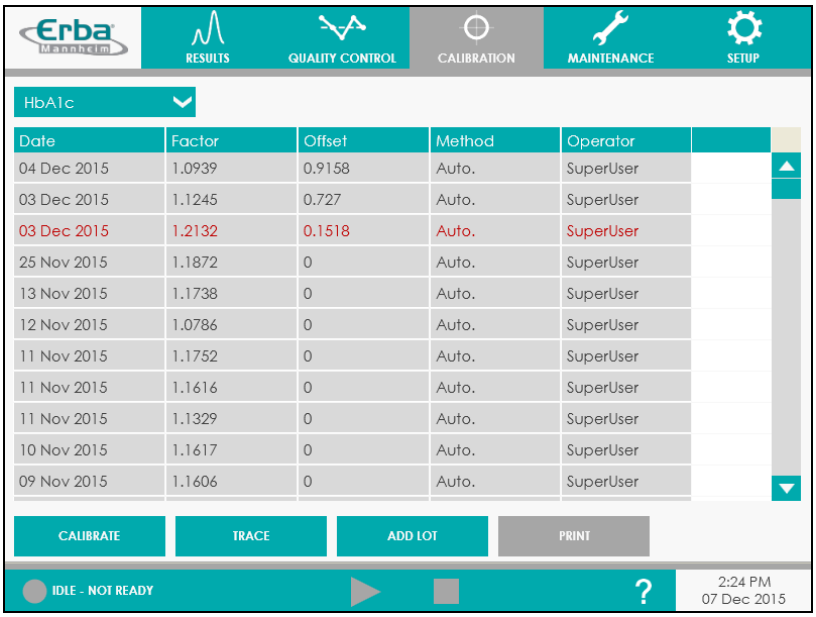
5.5.1. General information about Calibration reviews

- Calibrations are displayed from the most recent to the older ones
- The active calibration is displayed in **Bold Green** characters
- System rejected calibrations are displayed in Red characters
- Clicking on a calibration date will display the lines of all levels and determinations of this calibration procedure
- To view the details of the flags click on the flag logo to display that specific one : the flag detail area will be located at the bottom of the window, the selected point will be highlighted
- To view the details of another flag when the flag detail area is already open, just select the line you are interested in
- To close the flag details, just click on any flag logo
- If the TRACE button is clicked, the flag area will be automatically closed to make room for the visualization of the requested trace
- If more than 1 calibration are available for the same kit, it is possible to reactivate one, in this case a special Reactivate button becomes visible

5.5.2. Reviewing Calibrations

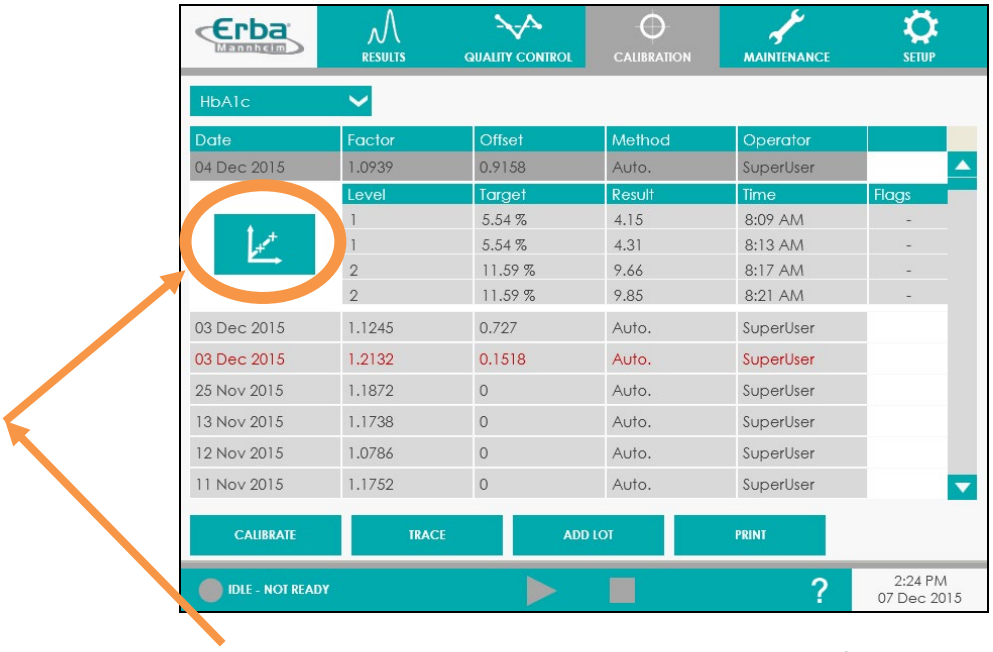
Once the calibration run is complete, to review the calibration:

1. Click on the Calibration Window button in the Top Menu.

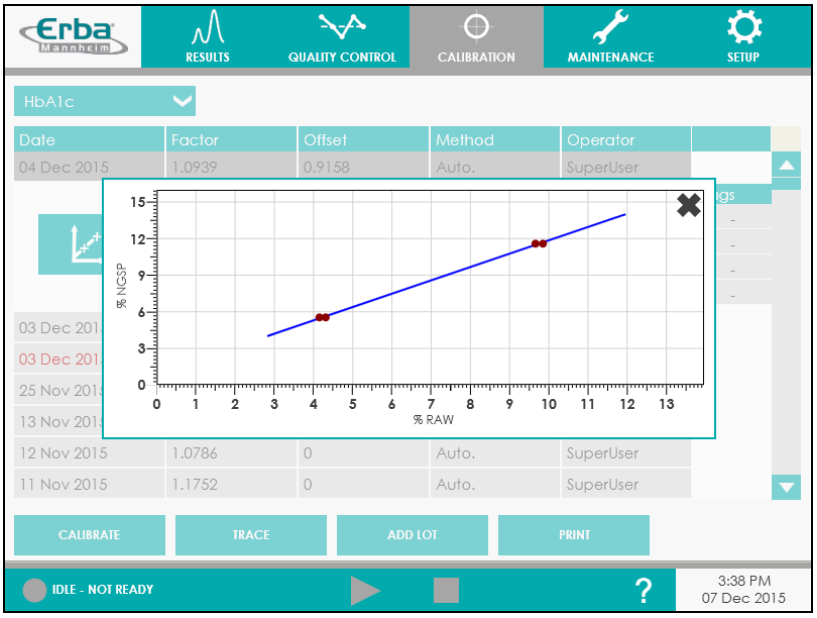


2. Calibrations are visible under a table format, *it displays the Factor and offset for this calibration, as well as the method used (automatic or manual) and the operator logged at the time the calibration was performed*

3. Click on a specific calibration row to review the target values and actual results for the high and low calibrators replicates performed during that calibration run. *It also includes the exact time of calculation of each test results.*

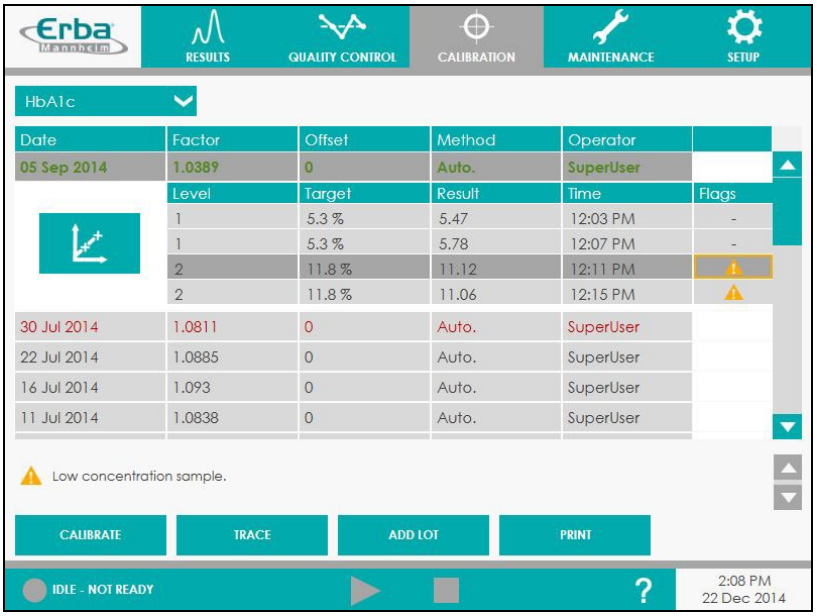


4. Press the Calibration Curve button to see the calibration curve for that calibration run. *The following window will open, displaying the % Raw results on the X axis versus the theoretical calibrator values:*



Clicking on the **X** will close the window to get back to the window underneath.

5. To review the details of flags mentioned for a determination, click on the Flag Icon ⚠. *The flag detail area will open and display the reason(s)*



5.5.3. Calibrator Trace Review

At its heart, the Hb-Vario is an ion-exchange HPLC instrument and as such its output is chromatographic data. The chromatography resulting from a single analysis is often called a 'trace'. Pressing the 'TRACE' button will allow you to review the chromatography for both the high and low calibrator samples. Reviewing it will allow inspection of the actual separation, Retention Times, and % area for all the different hemoglobin fractions. X axis = time in seconds, Y axis = m OD. It also displays the total area of the injection.

5.5.4. Reactivation of a calibration

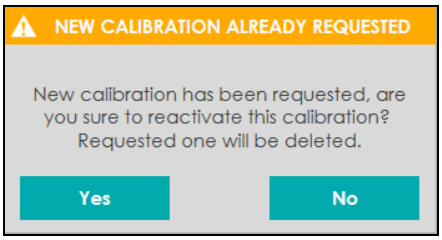
If more than 1 calibration exists for the same kit, it is possible to reactivate a former calibration curve. ***Only ADMIN rights users can reactivate a previous calibration, click here for more details.***

In this case clicking on the line of the non-active calibration (obtained with current kit), a special Reactivate button appears

Click on it

The calibration is reactivated and will be displayed in **Bold Green** characters.

In the specific case that the reactivation is requested when a Calibration has already been requested and is not yet performed, a confirmation message will appear:



5.6. Control Review

The final stage of a successful calibration is to ensure that the instrument is in control. This is done by reviewing the Control Sample results.

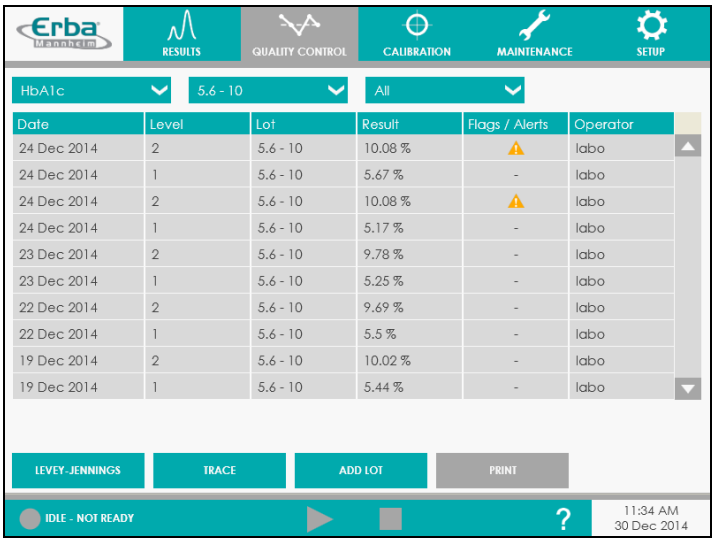
5.6.1. General information about Quality Control Review

- Quality Control results can be viewed under table format, or Levey Jennings graph
- From the table, the most recent quality control points are seen on top of the list, and older points are below
- By default the table opens with the HbA1c assay, the newest (longest expiry date) QC lot number, and all levels
- If the QC are within range there will be no Flag / Alerts icon
- A Flag icon does not necessarily mean that the QC determination is out of range (>2SD), but could be another system alert

- Levey Jennings can only display curves once only 1 specific level is selected, otherwise the graph will stay blank
- To view the details of the flags click on the Flag / Alerts icon to display that specific one: the details will be displayed immediately underneath the determination point
- To view the details of another flag when the flag detail area is already open, just select the line you are interested in
- To close the flag details, just click on any Flag / Alerts icon
- Unlike for calibrators, if the TRACE button is clicked, the Flag / Alerts area will remain open

5.6.2. Control Review from table

1. Begin by Clicking on the Quality Control button to open the QC window.

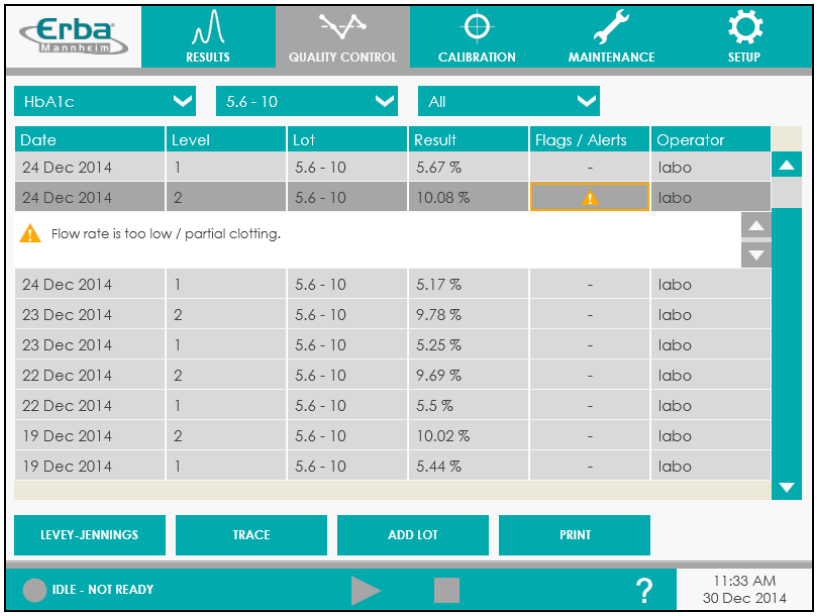


2. Review the last two control samples to ensure that they were successful.

5.6.2.1. View Flag /alerts details

If a **Flag / Alert is present**, a ⚠ will be present and can be clicked to check the cause(s) of the alert or flag. If the QC is within range, they will not display the alert icon.

- Click on the ⚠ to open the Flag / Alerts area immediately below the result



5.6.2.2. View Chromatogram (Trace)

To review the QC point **trace** (Chromatogram) highlight on QC point and click on the TRACE button

Figure 9: Trace display, with and without flag area

5.6.3. Levey Jennings review

5.6.3.1. To review the Levey Jennings graph:

1. from the Control results table (See 5.6.2)
2. select the assay, Lot number of control and a QC Level
3. Click on the LEVEY -JENNINGS button

5.6.3.2. General information about the Levey Jennings window

- The expected Mean is represented by a Black horizontal line on the Graph
- The 1SD limits are represented by a Green dotted line
- The 2SD limits are represented by an Orange dotted line (acceptable limits)
- The 3SD limits are represented by a Red line
- Each QC determination point is plotted on the graph at regular interval
- Each point is represented by a black full circle if its value can be represented inside the 3SD limits
- Each determination is joined to the next and previous ones by a teal color line
- If a point exceeds the 3SD limits it is represented by a black color filled triangle
- Each point is automatically taken into consideration for statistical calculation
- QC point(s) can be excluded from statistical calculation, they must be rejected to do so
- Rejected QC points are represented in Red filled color, and the graph lines no longer go through these points

5.6.3.3. To view the details of a specific point

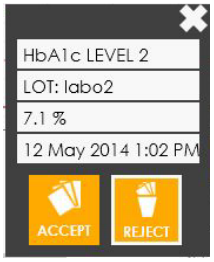
From the Levey Jennings graph:

1. Click on a QC point (Circle or Triangle), the Detail / Action window opens

2. View its details:
    - Name of the Quality Control material
    - Lot number
    - Value of the determination point and its unit
    - Date and Time of result

    An orange radio button is present to either Accept or Reject the QC point
3. Close the Detail / Action window by pressing the **X** on the upper right corner

5.6.3.3.1. To Reject a QC point



1. Click on the Reject button
2. Then close the Detail / Action window by pressing the **X** on the upper right corner
3. Only upon closing the detail window the graph will change and exclude the point

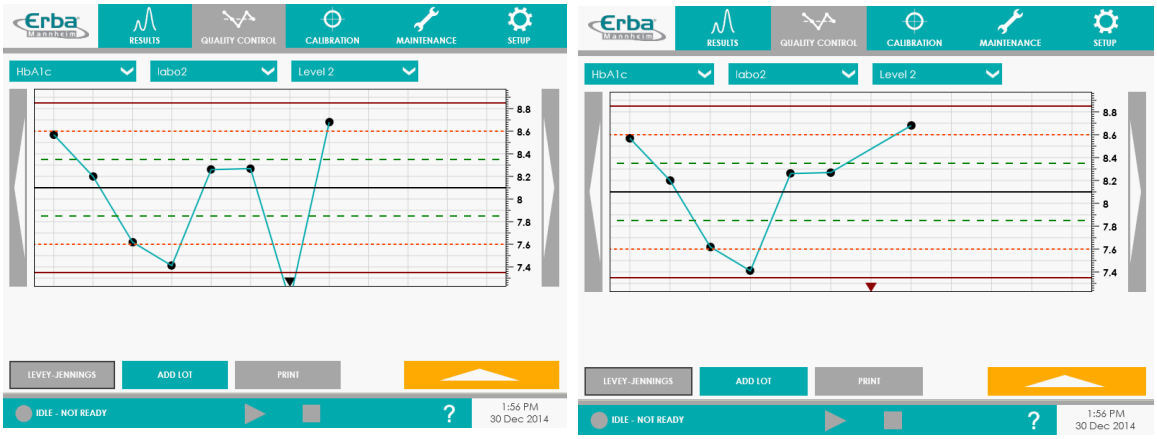
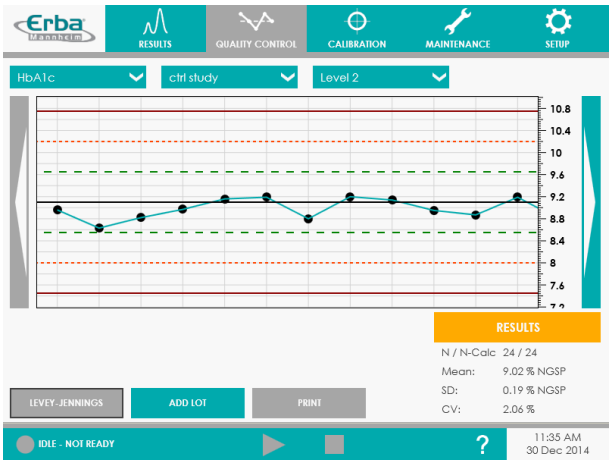


Figure 10: QC point exceeding -3SD: When accepted and when rejected

5.6.3.4. To view the statistical calculations

From the Levey Jennings graph:

1. Click on the orange arrow located at the bottom right corner of the Levey Jennings window
2. View the statistical calculation elements:
    - The N: Number of Quality Control Points for this specific level / Lot number
    - N-Calc: Number of Accepted Quality Control Points for this specific level / Lot number, used for the calculation
    - Mean: Calculated Mean and its unit
    - SD: Standard Deviation
    - CV: Coefficient of Variation in %



3. To close the statistics, click on the Orange Result label

6 Running

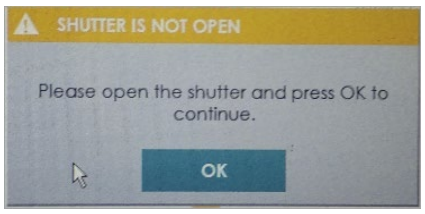
6.1. General Information about running samples

The system can be connected to the LIS, in which case the system will receive the information (sample name, test ordered, etc. from it)

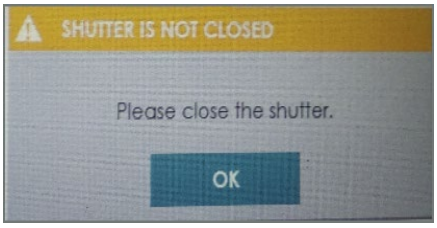
But it can also be configured to run one assay by default like HbA1c for example (See7.17 Setup). In which case when running with barcoded samples, the test request is extremely easy and fast.

To loading /unloading the sample racks, system will pop the messages to open / close the shutter

- To open the shutter following message will get displayed to open the shutter.



- Open the shutter, load the sample rack and click OK , sample rack holder will move to the loading/unloading position, ready to accept the sample rack.
- After loading /Unloading sample rack, following message will get displayed to close the shutter:



Running samples on the Hb-Vario can be achieved in different ways:

1. **Loading a rack of barcoded samples** and having the system scan for occupied spaces.
    a. If the system is connected to the LIS in bidirectional interface, then the requests will be retrieved from the LIS
    b. If the system is NOT connected to the LIS, then the system will propose to run all detected positions with the default assay and inform the user of all the unidentified positions
        i. The user can decide to let the system run as is
        ii. Or decide to change manually the orders and / or add new samples where barcodes could not be found.

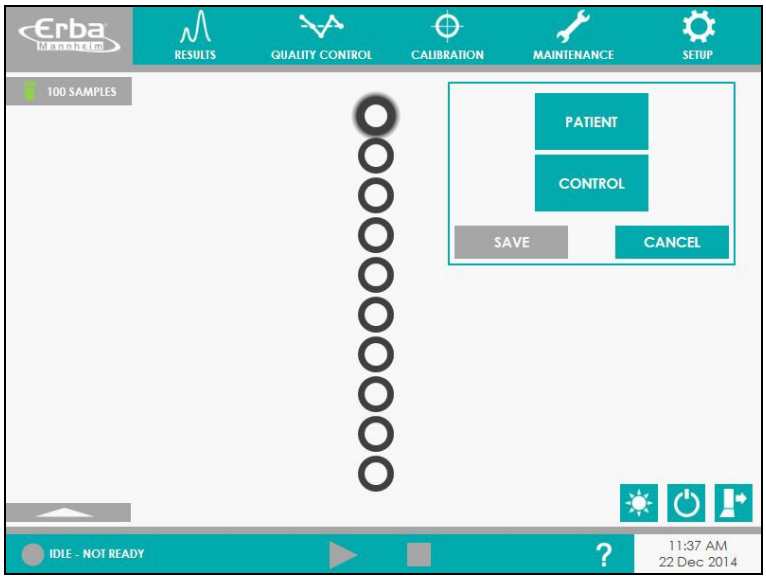
2. Registering each sample on the system before pressing the Run button and loading the rack into the system. The system will check for obvious differences between the sample information registered and what it detects and highlight discrepancies. Once any issues have been corrected the Run button can be pressed.

Option 2 is the one requiring more input from the user, thus it is the one described here in details. For laboratories using barcoded samples, jump to 6.5 loading samples.

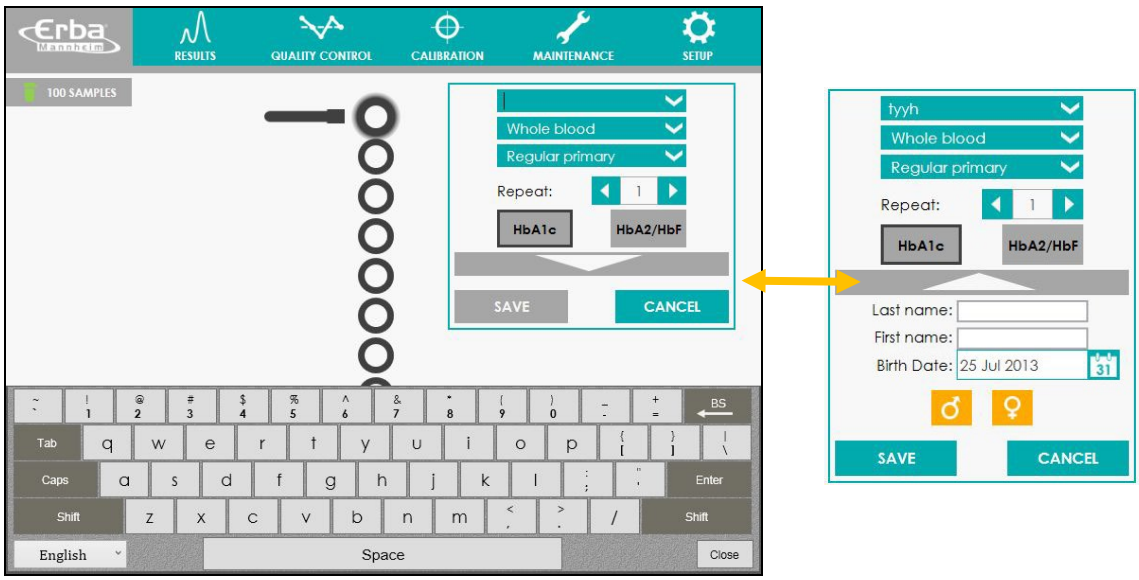
6.2. Registering Samples

To register samples on the system:

1. Eject the rack holder so it comes in loading/unloading position by clicking on 
2. From the Status window 
3. Press an Empty Sample Space Icon
4. Select 'PATIENT' from the Patient / Control dialogue



5. At the Patient Data Entry dialogue enter the required information and provide any supplementary information in the lower section, clicking on the grey expander button as required.



6. Press SAVE once all of the required information has been entered.
7. Repeat this process until all of the samples intended for the run have been registered.
8. Proceed to the loading the samples on the rack before pressing the Run button  (see 6.5 Loading samples for more details)

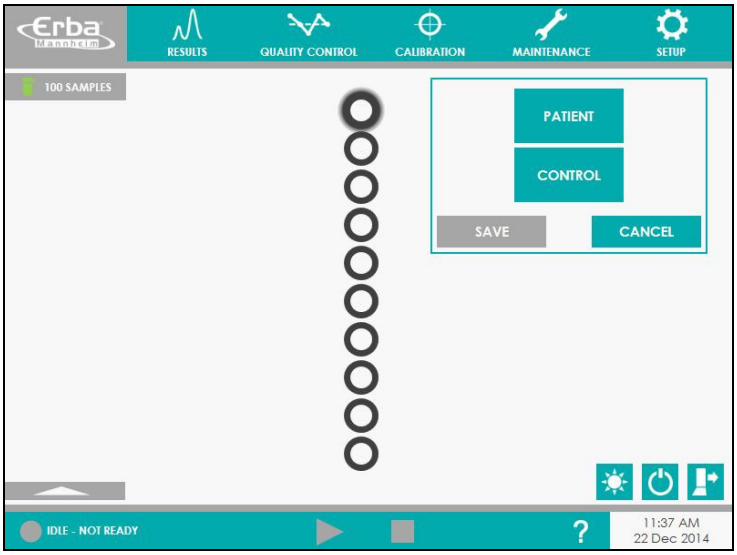
Note: Default information is already prefilled: Assay name, Whole Blood, Tube size, number of repeats. Practically, only the sample ID can be entered (typed or scanned with an external USB barcode reader). All prefilled information can be modified as needed

6.3. Registering Controls

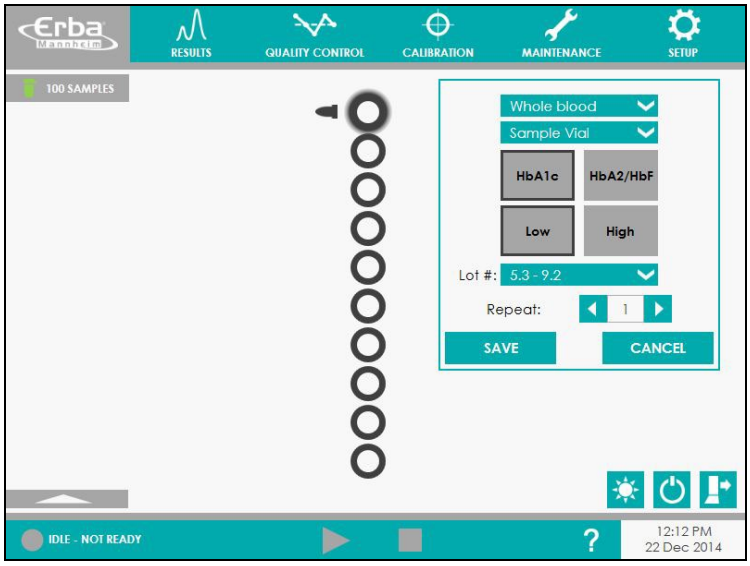
In case the run does not follow a calibration procedure which includes the Quality Control material, controls should be run on a regular basis. Each laboratory should follow its own protocol for frequency of QC run. It is recommended to run at least 2 levels of QC per 24 hour of activity to check the system performance. QC can also be added any time as felt needed.

To register Controls on the system:

1. With the rack holder in loading/unloading position (press  if not in position)
2. From the Status window 
3. Press an Empty Sample Space Icon
4. Select ‘CONTROL’ from the Patient / Control dialogue



5. Select the QC level
6. Select the QC lot from the available list
7. Press the SAVE button



Note: Default information is already prefilled: Assay name, Whole Blood, sample vial, number of repeats. Practically, only the QC level and its lot number have to be entered.

All prefilled information can be modified as needed

The lot number will not display available list until the QC level is selected

6.4. Preparing Samples

The Hb-Vario will run whole blood and pre-dilute (Lysate) samples in any of the tube types mentioned in section ***7.6.1.***

Sample receptacles which contain **whole blood** must contain the following minimum volume of material:

- 13 x 75 mm Primary Tube : 300µl
- 13 x 100 mm Primary Tube : 300µl
- Microcapillary Tube : 50µl
- 1.5 ml Sample Vial : 50µl

Sample receptacles which contain **pre-dilute** (**lysate**) sample must be prepared at a concentration of 25 µl of whole blood per 1 ml of Hemolyser or 50µl + 2ml. Furthermore, these receptacles must contain the following minimum volumes of pre-dilute material:

- 13 x 75 mm Primary Tube : 1000µl
- 13 x 100 mm Primary Tube : 1000µl
- Microcapillary Tube : N/A
- 1.5 ml Sample Vial : 700µl

6.5. Loading Samples

Warning: Samples, loaded in Microcapillary tubes (Sarstedt Microvette® type) or 1.5 ml Sample Vials *(this includes Eppendorf 1.5ml or microcapillary collected in brands other than Sarstedt)* must be placed into the correct adaptor provided with the instrument (Respectively the high magnet and the low magnet adaptors). This includes Control and Calibrator samples in Eppendorf with the low magnet adaptor. Usage of the wrong adaptor **may lead to damage to the probe assembly**.

6.5.1. Adaptors

There are 2 types of adaptors for loading samples into the rack.

Adaptor	Name	Intended use
	Top magnet adaptor	• **Exclusively for the EDTA Microvette® Sarstedt microcapillary collection tubes.** • *This includes the Microvette® 100µl and 200µl EDTA microcapillary collection tubes and the Microvette® 500µl EDTA*
	Bottom magnet adaptor	• **Eppendorf 1.5ml** • **Sample vials that sit on the adaptor resting base** • **microcapillary collection tubes OTHER than Microvette® Sarstedt brand**

6.5.2. Tubes

Below are descriptions of the tubes that can be used on the Hb-Vario.

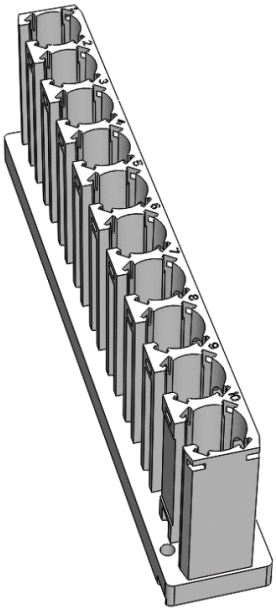
Tube image	Description	Tube type setting (in Software)	Use on rack
	13 x 100 mm primary tube	Tall Primary	place directly on the sample rack (uncapped)

	13 x 75 mm primary tube	Regular primary	place directly on the sample rack (uncapped)
	Primary Capillary collection tubes Microvette® Sarstedt,	Microcapillary	Place over a top magnetic adaptor (uncapped)
	Other brands of primary capillary collection systems ***(that sit on the resting base of the adaptor)***	Sample Vial	place over a bottom magnetic adaptor (uncapped)
	Sample vial, Eppendorf® type 1.5ml	Sample Vial	place over a bottom magnetic adaptor (uncapped)
	Other types of Sample vial, ***where the bottom of the vial sits on the resting base of the adaptor***	Sample Vial	place over a bottom magnetic adaptor (uncapped)
	13 x 75 mm transfer tube	Regular primary	place directly on the sample rack (uncapped)

6.5.3. Sample Rack

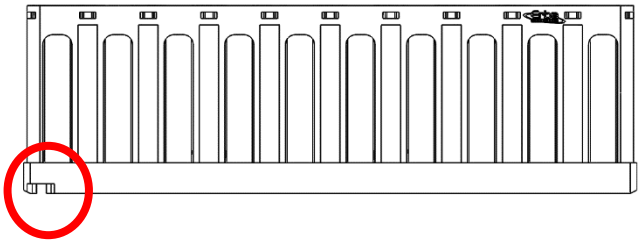
The rack is not symmetrical and needs to be inserted correctly onto the rack holder in the instrument.

The rack is as follows:

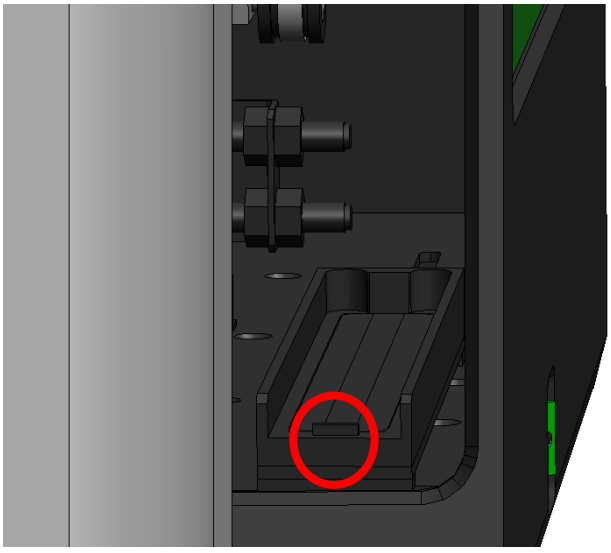


As seen on the image on the left, the rack is open on one side from bottom up; this side should be oriented towards the left of the instrument

The barcodes of the sample tubes should be oriented towards this side as well to allow the barcode reader to read correctly



As seen on the image of the rack, there is an indentation at the bottom of the rack to position correctly the rack onto the holder.

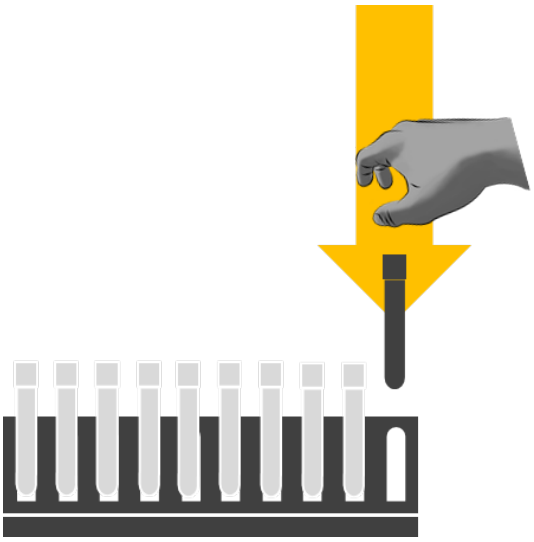


On the rack holder, an index positioning pin should be matched with the indentation of the rack.

The rack should be pushed all the way to the rear of the rack holder.

Figure 11: Examples of tube loading, with respective adaptors: 1 =13x75mm transfer tube = regular primary, 2 = 13x75mm primary = regular primary, 3 = Eppendorf 1.5ml = sample vial, 4 = Microvette® primary = Microcapillary, 5 = Primary microcapillary other than Microvette® = sample vial, 6 = Other type of sample vials = Sample vial,7 = 13x100mm primary =Tall Primary

6.5.4. Loading

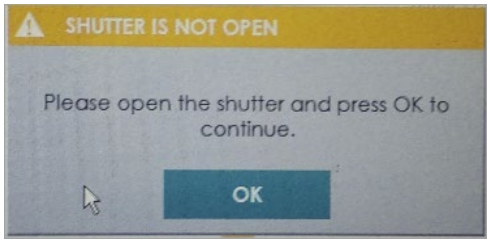


Warning: Be sure to **remove** all sample tube / vial **caps** before inserting the samples into the system. Sampling from capped tubes / vials will result in damage to the sample probe.

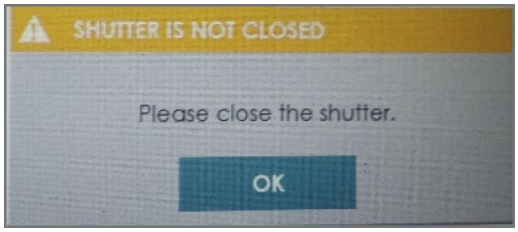
1. Load the tubes :
   - **As registered** on the system into the Hb-Vario's sample rack ensuring that the correct tube type is used and that each sample is loaded in the registered position.

- **Or load the barcoded unregistered tubes**, barcodes facing to the left into the Hb-Vario's sample rack

- To open the shutter message will get displayed to open the shutter.



- Open the shutter and click OK, sample rack holder will move to the loading/unloading position, ready to accept the sample rack.
- After loading /Unloading sample rack, message will get displayed to close the shutter.

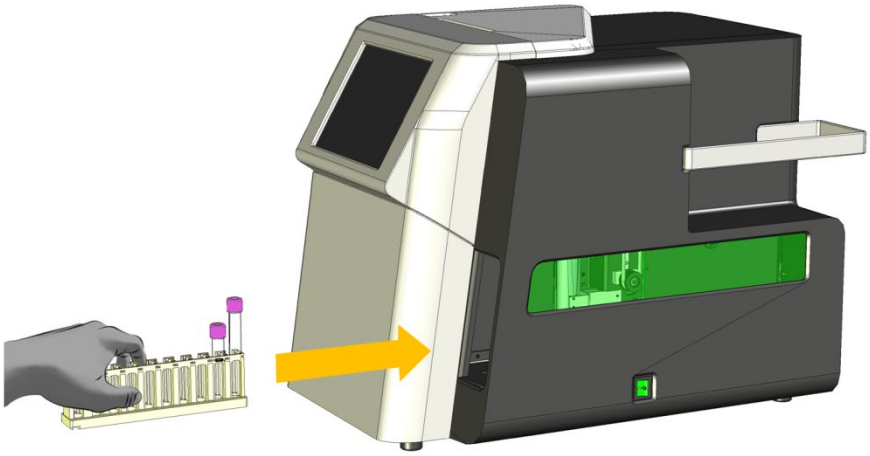


2. Place the loaded rack into the rack holder.

   a) For this the rack holder needs to be in the loading/unloading position. Press the  button to get the rack holder into the correct position if not already there.

   b) Make sure the rack is presented in the correct orientation (the rack opening pointing to the left). Barcode facing to the left. Push the rack all the way into the holder. So that rack front extremity is matching the rack holder extremity. (see 6.5.3 sample rack for more details)

3. Press the  button to have the Hb-Vario accept the rack holder and the loaded rack ready for analysis.



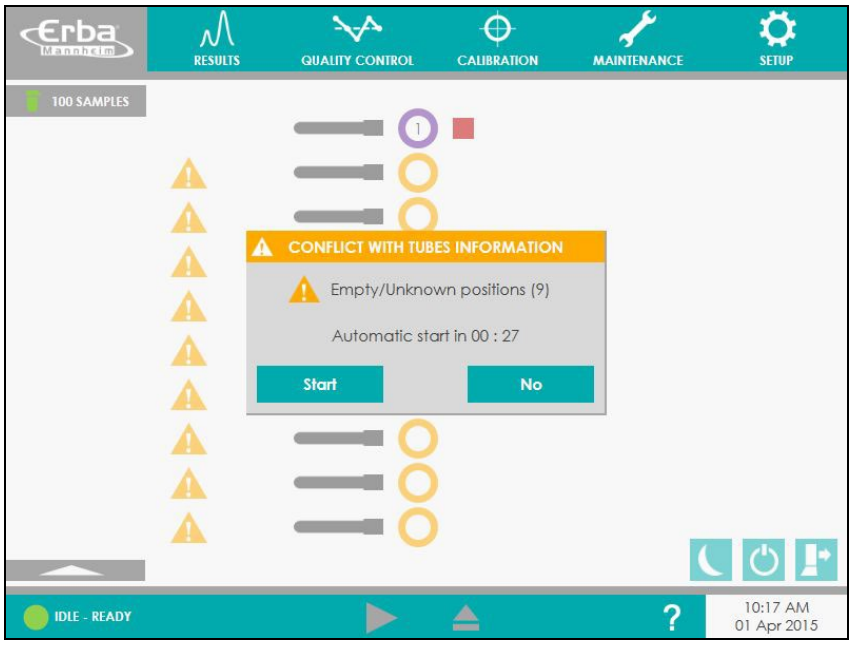
The system will scan the rack and check the occupied positions against the registered data before highlighting any inconsistencies.

It includes the adaptors detections (top or bottom magnets matching). Any position of the rack where the instrument did not read a barcode or for which no request have been manually entered will be highlighted as an unknown sample.

Note: inconsistencies can be:

- Unknown positions which will be flagged as
- Mismatch of barcode between manually defined and automatic barcode reading
- Conflict of vial size from manual definition and auto sensing of magnetic adaptor will be solved automatically by the automatic detection of the magnetic sensor, if no sensor detected then by default the Regular Primary will be used.

4. The system will give a countdown of 30 seconds before starting the run automatically



a) The run can start on its own after the 30 second countdown is elapsed

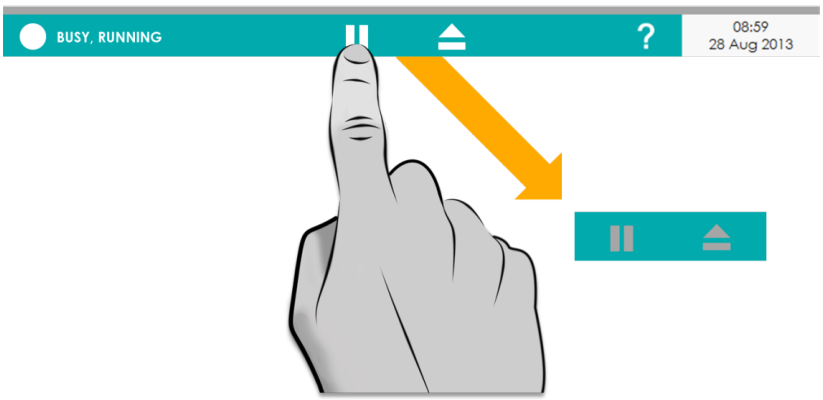
b) The run can be forced to start immediately (not wait for the 30 seconds) by pressing Start

c) The rack can be corrected before the 30 second wait period by clicking on No
   - Selecting the sample with inconsistencies and then pressing the Selected Sample Data area allows them to be resolved.

- Once inconsistencies are resolved, press the Run button to have the system analyze the samples.

6.6. Pausing the Run

Once the run begins, the Pause button will replace the Run button. The Pause button will be white to indicate that it is active and can be pressed. Pressing the Pause button during the run will allow the samples currently being analyzed and prepared to complete. Once pressed the Pause button and the Eject button will become inactive until the pause is complete.



6.7. A Paused Run

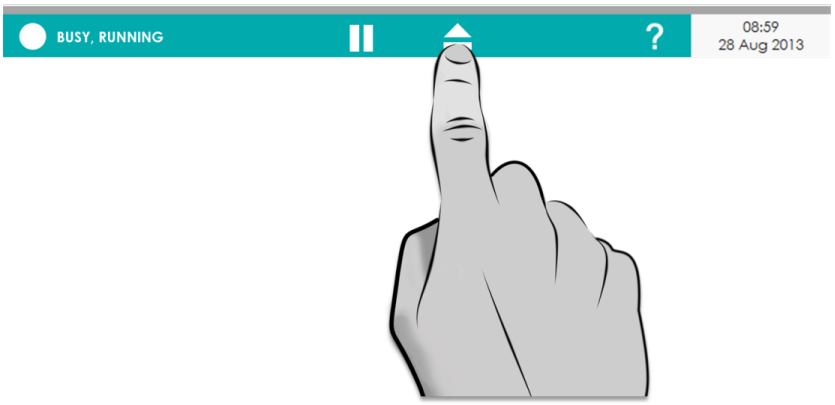
Once a pause has been successfully completed, the Eject button may be pressed. Pressing Eject whilst in pause automatically removes completed samples from the system registry. The system will then ask whether the remaining, incomplete samples, are also to be discarded.

Note: Discarding incomplete samples effectively ends the run.

The advantage of a Pause is that it does not interrupt the system cycle and it allows samples to be added or removed before the rack is complete.

6.8. Emergency Stop

Whilst the Pause button brings the system to an orderly stop, without sacrificing samples currently being analyzed or prepared, **pressing the Eject button during a run is interpreted as an emergency stop.** The system will first ask for confirmation of this action but once confirmed it will immediately abandon the run. The samples being analyzed or prepared will be lost, the rack holder will eject and all of the registered tube information will be lost.



Note: Any results which have been completed during the run, before the emergency stop, will be retained.

6.9. Reviewing Sample results

Reviewing sample data is done under Results. For further information on reviewing sample data refer to section 7.8

6.10. Printouts

Results will automatically print if the Auto Printing is activated from Setup (See Printer)

Otherwise printouts can be requested from Results, Print button (see Print Results)

6.10.1. Printouts of patient results

Printouts of patients can be configured from the Setup, Printer for selection of data to be printed. (See Printer).

2 different reports exist:

- A complete report
- A short report

The 2 different reports are presented below.

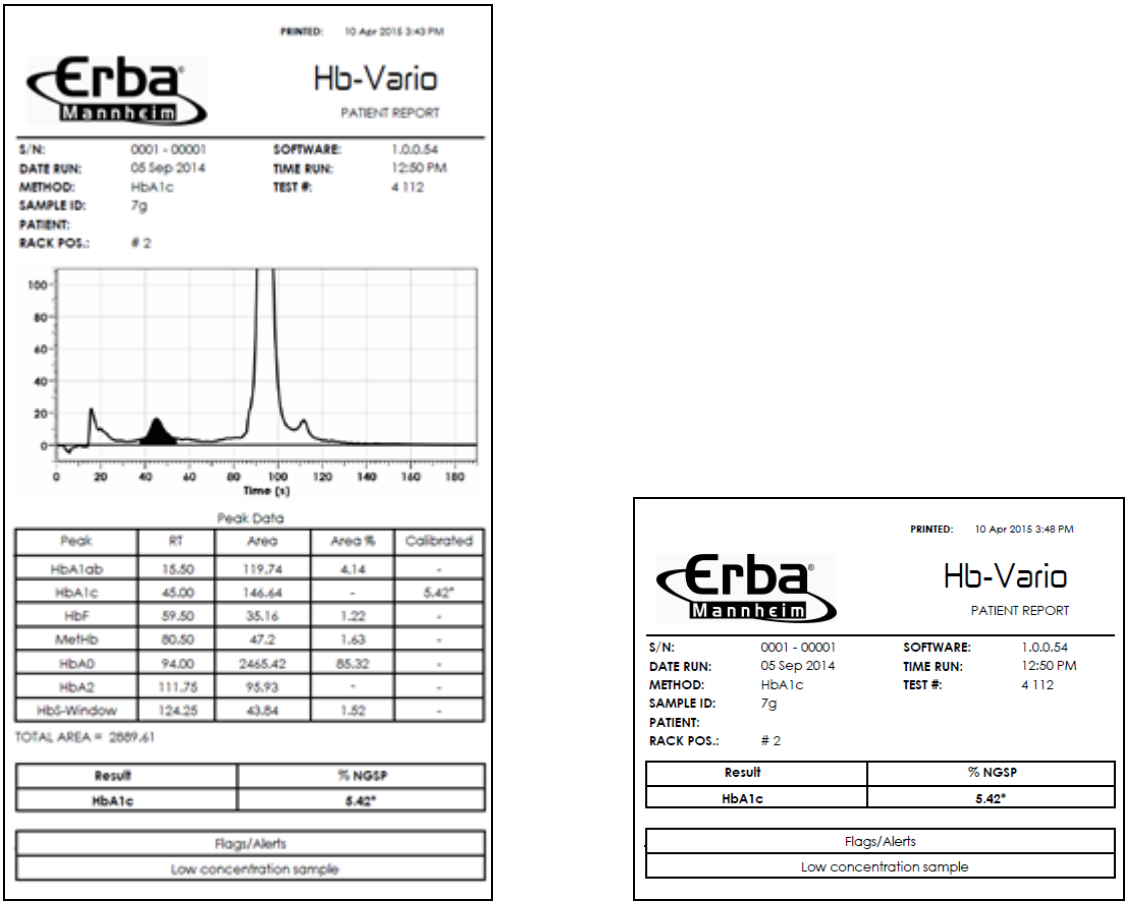


Figure 12: Printout of patient test (complete report left, Short report (right)

6.10.2. Printouts of calibrators or controls

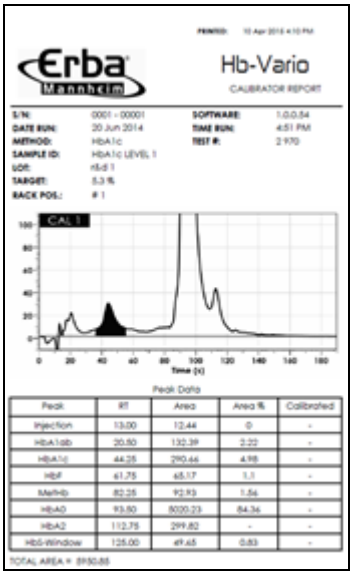
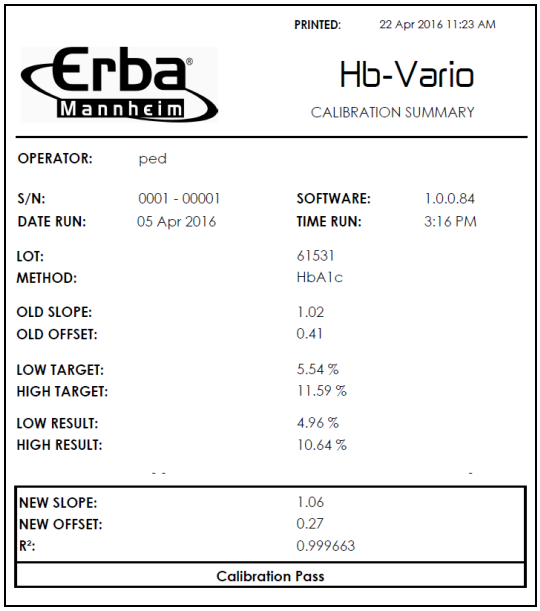


Figure 13: Printout of a CALIBRATOR REPORT *(CONTROL REPORT is arranged in the same manner)*

6.10.3. Printouts of calibration reports

At the end of a calibration procedure, a calibration report is printed, as follows.



PRINTED: 22 Apr 2016 11:23 AM

Erba Mannheim

Hb-Vario

CALIBRATION SUMMARY

OPERATOR:	ped		
S/N:	0001 - 00001	SOFTWARE:	1.0.0.84
DATE RUN:	05 Apr 2016	TIME RUN:	3:16 PM
LOT:		61531	
METHOD:		HbA1c	
OLD SLOPE:		1.02	
OLD OFFSET:		0.41	
LOW TARGET:		5.54 %	
HIGH TARGET:		11.59 %	
LOW RESULT:		4.96 %	
HIGH RESULT:		10.64 %	
NEW SLOPE:		1.06	
NEW OFFSET:		0.27	
R^2:		0.999663	

Calibration Pass

Figure 14: Calibration report printout

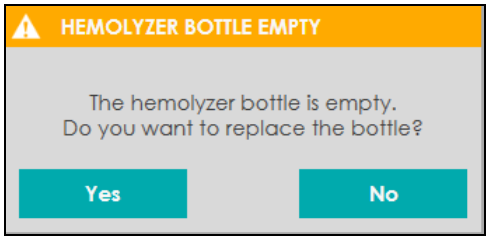
6.11. Running out of kit components

The Hb-Vario continuously verifies the volumes being consumed and the possible number of tests which are remaining. Before or during a run, it is therefore possible that the number of possible tests is lower than the requested tests. Below are the different possible scenarios and behaviors of the instrument in each instance.

Note: it is possible to reinstall a kit that has not been consumed fully of its reagents content. See 6.12 for more details.

6.11.1. Hemolyser bottle is empty

The Hemolyser is what is most consumed during rinses, and startups. Therefore, for low workload, the proportion of Hemolyser consumed is greater, and it is possible that the Hemolyser bottle will be consumed before the 100 test pack/column is consumed.
In case the Hemolyser runs out during a run, the instrument goes in Pause, and opens a pop up message:



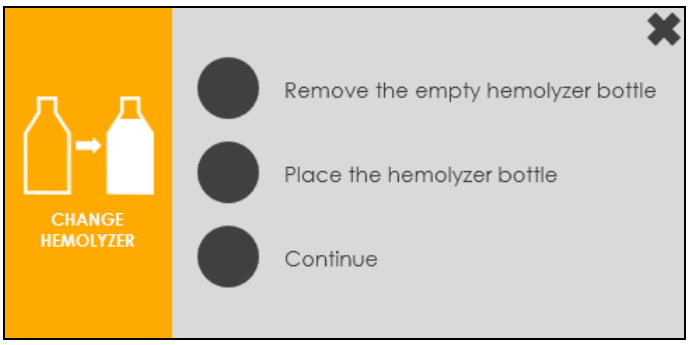
Click on the NO button to cancel and close the window without replacing the Hemolyser bottle.

In this case the system will remain in PAUSE. Resuming run will reopen the same pop up for later replacement of the Hemolyser. Otherwise the whole kit can be replaced before consuming the entire current kit. To reinstall a kit later, see 6.12 for more details.

Otherwise, to replace the hemolyser bottle:

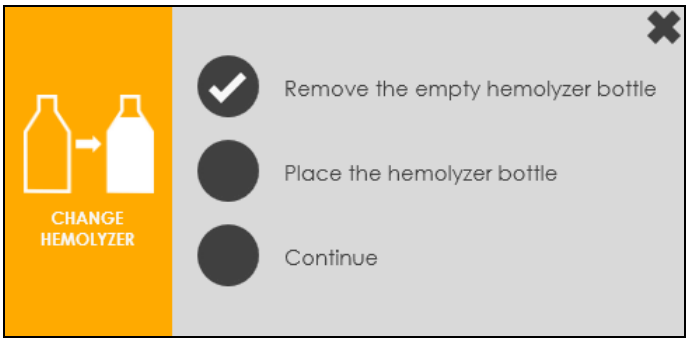
1. Click on the YES button

Another instruction window will open and give step by step instructions to replace the Hemolyser bottle.



Follow the on-screen instructions until completion, *or close the window by clicking on the X on the top right corner*:

2. Remove the empty hemolyzer bottle, then click on the corresponding circle check box.



3. Place the new and full hemolyzer bottle, then click on the corresponding circle check box
4. Then click on continue

The system will update its Hemolyser content to the new full bottle filling volume and close the window automatically and go back to the PAUSE status.

Warning: it is important to replace the bottle **ONLY with a brand new full bottle**, and not a partial bottle as the system will consider the count down from a full bottle. DO NOT refill the kit bottle with left over Hemolyser as it cannot guarantee the integrity of the liquid, leading to possible hydraulic contaminations, blockage, performance issues, erroneous results or depriming.

6.11.2. Insufficient kit remaining tests

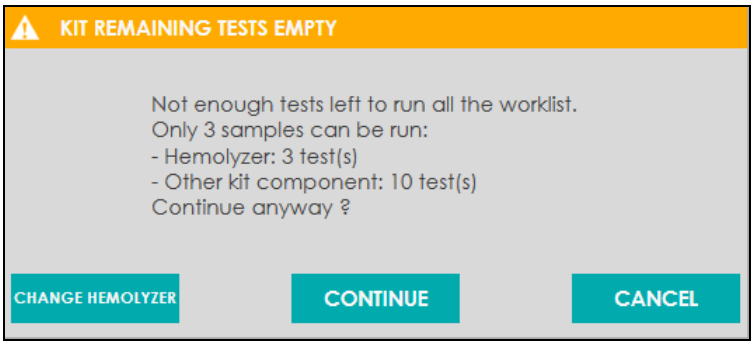
When starting a run, the system compares the requested tests with the remaining tests and components. In case the system detects insufficient supply, a message will be displayed. It can be of different scenarios:

1. Insufficient Hemolyser and reagents/column counts
2. Insufficient reagent/column counts
3. Insufficient hemolyser

The case 3 was already explained in the point 6.11.1, with the exception that once the "change Hemolyser" procedure is completed, the run starts automatically instead of placing the instrument in PAUSE.
The cases 1 and 2 will be detailed below.

6.11.2.1. Insufficient Hemolyser and other kit components

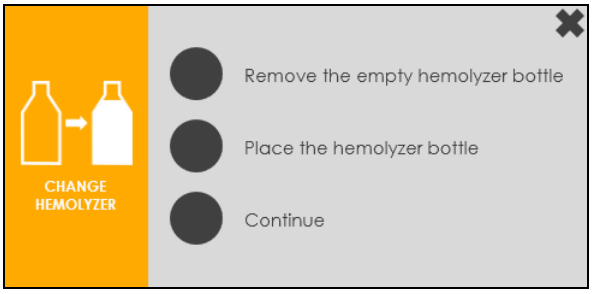
In case of Insufficient Hemolyser coupled with insufficiency of other combined components/ counts, the following pop up message appears, displaying the specific counts:



There are then 3 possible courses of actions:

1. **Change Hemolyser**: to replace the Hemolyser and finish the kit till depletion. The full run will not be possible but no kit test will be wasted
2. **Continue**: to start the run immediately and up to the possible tests stated in the pop up. Up to this number, the kit will either be empty and you will be instructed to replace it or the hemolyser will be depleted and you will be requested to replace the hemolyser.
3. **Cancel**: to close the window and proceed to a kit replacement. For this option, after the window is close, proceed to a regular kit replacement in the maintenance menu.

In case 1, when clicking on the Change Hemolyzer button, the change Hemolyser instruction page will open

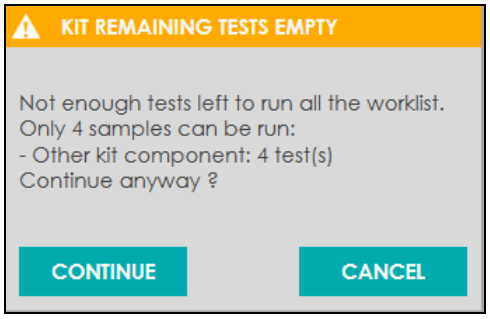


Proceed as explained in 6.11.1. When the change hemolyzer window closes, the run will start automatically.

Warning: it is important to replace the bottle **ONLY with a brand new full bottle**, and not a partial bottle as the system will consider the count down from a full bottle. DO NOT refill the kit bottle with left over Hemolyser as it cannot guarantee the integrity of the liquid, leading to possible hydraulic contaminations, blockage, performance issues, erroneous results or depriming.

6.11.2.2. Insufficient kit components other than hemolyser

In case there is not enough components left in the kit to run the entire programmed list of tests, the following window pop up will open:

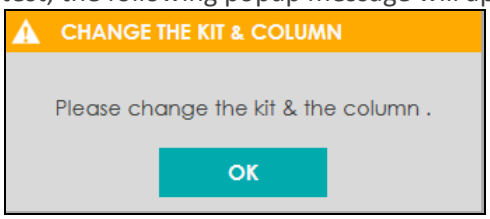


2 options:

1. **Continue**: will continue and start the run with the remaining available components, up to the number of tests stated in the popup window.
2. **Cancel**: will close the pop up message and place the instrument in PAUSE. Afterwards, it is possible to replace the kit by going to Maintenance, Change kit & column, See 7.16.6 for more details.

6.11.3. Replace reagent kit message

During a run, when the number of possible tests gets to 0 or when the reagents A and/or B are no longer sufficient to perform a test, the following popup message will appear:



Click OK to close the window.
Then proceed to Maintenance, Change kit & column. See 7.16.6 for more details.

6.12. To reinstall a kit that was not fully consumed

Sometimes, the user may choose to replace a kit before it is fully consumed. It is then possible to reinstall it to use the left over kit/components.

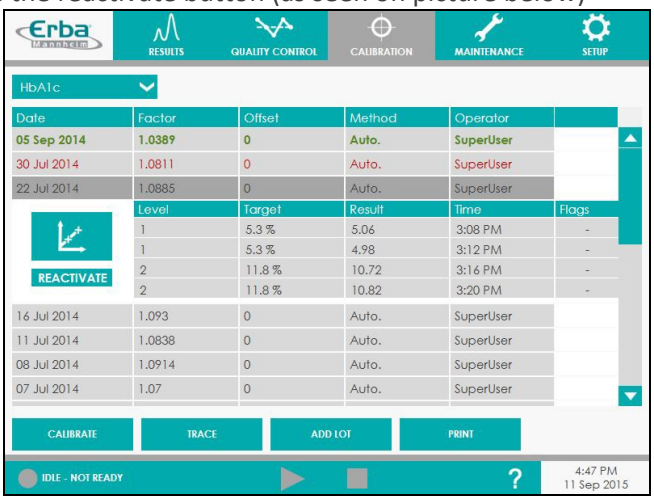
When removing a partially used kit:

- Make sure that the column is correctly placed back in its humid storage cup
- Label the column container with a code to identify this column
- Place it in the fridge until it can be reinstalled in the analyser.
- Make sure the reagent pack containing the open Hemolyser, Reagent A and Reagent B is labeled with the matching code of the column (to insure reinstallation of the correct matching set).
- Make sure to correctly and tightly recap all 3 bottles
- Place the reagent pack in a refrigerator with its matching column until the time of reinstallation.

When reinstalling a partially used kit:

- Make sure the reagent pack is taken out of the fridge about 30 minutes before reinstalling to equilibrate the solutions to room temperature.
- Insure to reinstall the correct matching column and reagent pack (matching code labeled at the time of removal/storage). *The system has specific settings for this column/reagent pack. Failure to do so may induce wrong retention times, and wrong results due to incorrect background.*

- Then proceed to Maintenance, Change kit & column and follow the steps as for a new kit. See 7.16.6 for more details.
- You will be required to reactivate the last valid calibration for this kit prior to resume testing, or calibrate the kit if it was never successfully calibrated:
- To do so, go to Calibration and click on previous black lettering calibration records until you find one that offers the reactivate button (as seen on picture below)



See 5.5.4 for details on calibration reactivation

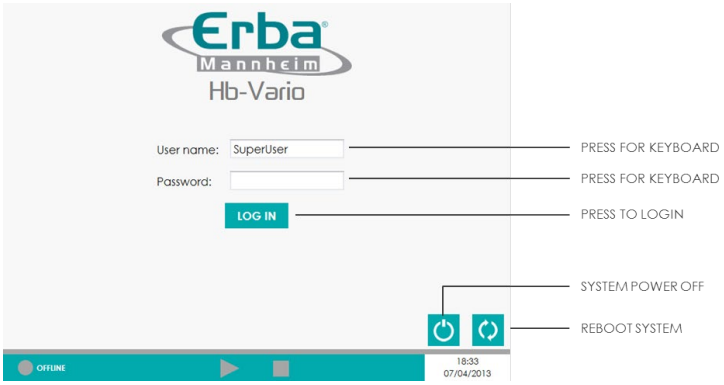
Note: Limitations in reinstalling a partially used kit:
The kit needs to be within its expiration limit
The kit needs to be within its stability limit (maximum 30 days after its original installation Date)

7 User Interface References

This section provides a complete screen by screen walkthrough of the software's User Interface (UI) in order to fully orient the user.

Read First: It is recommended that this section be read prior to further operation of the system.

7.1. Login Screen



7.1.1. User name Field

The user name corresponds to either an individual user or a group of users e.g. lab technician, or supervisors. New user names can be added to the instrument via the Settings Window, by authorized users. Pressing this field will display the on-screen keyboard to allow data entry. A maximum input of 20 characters is possible.

7.1.2. Password Field

Passwords must match to the corresponding User name entered. Pressing this field will display the on-screen keyboard. A maximum input of 20 characters is possible.

7.1.3. LOG IN Button

Once a User name and Password have been entered into the relevant fields, the 'LOG IN' Button will become colored and can be pressed. Passwords and User names will be checked and if correct the system will grant access to the rest of the instrument's functions.

7.1.4. Power Off Button

Pressing the Power Off Button will switch off the PC portion of the system, shutting down Windows properly so that it is safe to switch off the power completely using the button on the right side panel. Use this when wishing to power off the analyzer for extended periods of time.

Warning: Never press the power button, and cut power to the instrument, without first pressing the Power Off button in the User Interface.

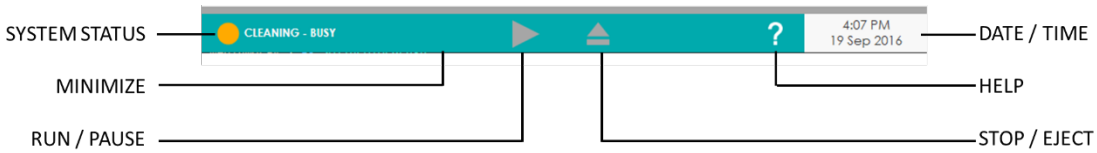
7.1.5. Reboot Button

The Reboot button should be used when only a system restart is required of the PC portion of the analyser.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Reboot (computer)		There is no inactive status for this button	This button is used to restart the computer. Once the computer restarted, it will automatically come back to the Login window. *The Login window is accessible by clicking on the Log out button only from Not ready / ready / standby modes*	Button

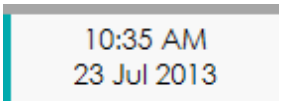
7.2. Bottom Menu

The Bottom Menu is visible in all Windows and provides current date and time along with the current status of the instrument. It also provides access to two key controls.



7.2.1. Date and Time Display

Date and Time are displayed according to the settings in the setting screen and can be changed to some degree by users with sufficient access.

AREA	DEFAULT / ACTIVE STATUS	DESCRIPTION / USE	FORMAT
DATE / TIME		This area displays the time and date of the computer. The format is forced to display the date in day Month in abbreviated letters from the regional setting	information field updated in real time

7.2.2. System Status Icon

The System Status Icon allows you to see at a glance, what the status of the instrument is from any of the analyser's sub windows. The color of this icon changes along with the description. These status and colors changes are as follows:

ICON	INSTRUMENT ACTION	INSTRUMENT STATUS	DESCRIPTION / USE	FORMAT
	IDLE	NOT READY	A Startup needs to be performed before it can go to the ready status	Information
	STARTING UP	BUSY	The instrument startup cycle is in process	Information
	IDLE	READY	The instrument is ready (Startup completed, ready to run)	Information
	RACK LOADING	BUSY	The rack loading is in process, wait for the end of the cycle to continue with the process	Information
	RUNNING	BUSY	The instrument is testing	Information
	PAUSING	BUSY	The instrument is Pausing, from the moment the pause button is pressed and pause request is confirmed to the moment the last result of the run is completed	Information
	SHUTTING DOWN	BUSY	The instrument is processing to the standbymode	Information
	IDLE	STANDBY	The instrument is in Standby mode. To resume testing, just load a rack and press run	Information
	CLEANING	BUSY	The instrument is processing a cleaning cycle. Wait for the end of the cycle to have access to the system	Information
	PRIMING	BUSY	The instrument is processing a priming cycle. Wait for the end of the cycle to have access to the system	Information

7.2.3. Run / Pause

Depending on the current status of the instrument, this button will be displayed as either a classic right facing, Play, triangle or as two vertical bars, Pause. The Play / Run button tells the unit to run whatever samples have currently been added to the rack. The Pause button stops the system from running any further samples from this run but allows it to properly complete the samples it is currently analyzing (see sections 6.6and 6.7 for more details on this function).

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
PLAY			Press Play to start the run. When running the button toggles to Pause Active The Play button is inactive when the instrument is NOT READY	Button toggling between play and pause depending on the analyser operation status
PAUSE			Press pause to not initiate new sample aspirations during a run (the started tests will be completed till results reporting) During the time of completing the started tests, the Status of the instrument becomes PAUSING /BUSY During that time the button is inactive. Once the last sample started is completed the rack can then be ejected. Completed samples will be erased from the sample tray, a question will be asked to conserve the unfinished samples / tests programmed. Answering No will erase the whole sample tray programming. Answering Yes will keep all incomplete requests. No tests can be added to the unoccupied positions.	Button toggling between play and pause depending on the analyser operation status

7.2.4. Stop / Eject

Depending on the current status of the instrument, this button will be displayed as either a square, Stop, button or as the classic triangle on top of a bar, Eject, symbol. The Stop button is only visible during a Startup or Standby cycle to prevent the unit from completing this cycle. The Eject button is used to eject the rack holder from the system. If pressed during a run, pressing this button will immediately stop the run (see section 6.8 for further details on this).

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
STOP			This button is present only during a Startup or Standby cycle to interrupt them. Once the cycle is completed, the button toggles automatically to the Eject button	Button toggling between Stop and Eject
EJECT			This button is used to eject the rack holder If pressed during a run it is interpreted as a stop. After confirmation all started samples will be interrupted before result and all programmed tests will be removed from the rack	Button toggling between Stop and Eject

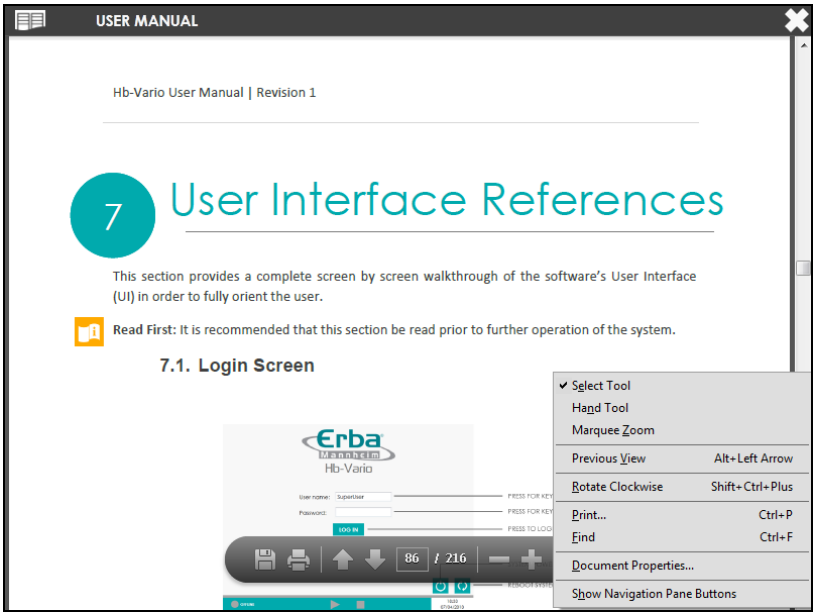
7.2.5. Help

The help button will open the user manual at the specific section where a brief description of the window from which the Help button was clicked to help user's orientation.

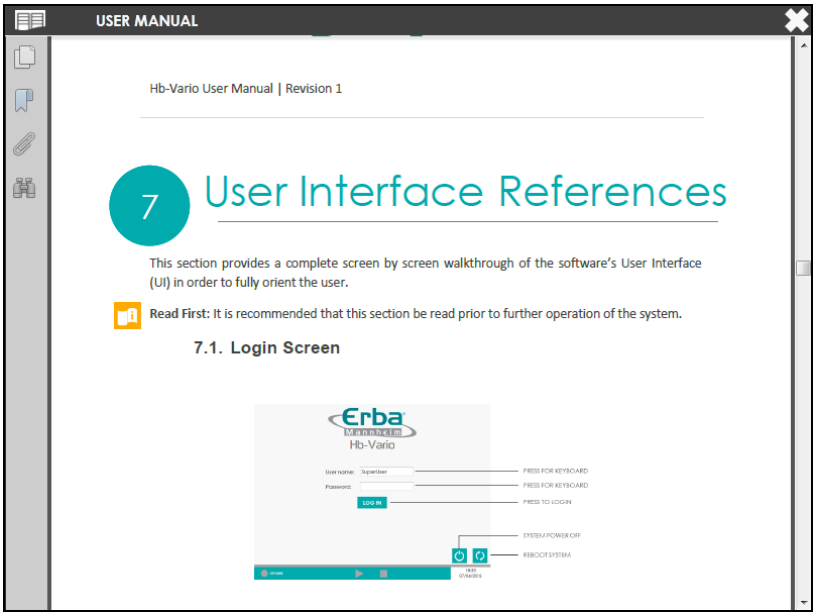
BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
HELP		Always active	This button is opening help files in relation to the current screen	button

The user manual once open can be browsed using Adobe Reader functionalities.

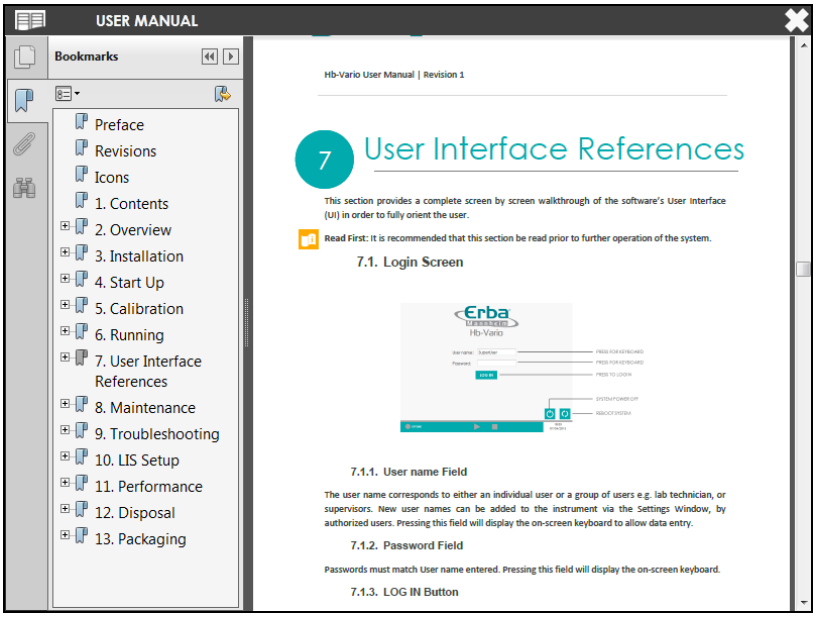
1. With your fingers, a long pressure on the User manual screen, will display the right click options:



2. Click on the "Show Navigation Pane Buttons" option to activate the Navigation bar



3. Once activated, click on the  icon to display the chapter titles and facilitate the browsing of the user manual



4. Click on the + sign in front of a title to develop the sub-menus
5. Click on a title/sub title to go directly to that section.

Note: The  icon (Search) allows to search by key-word, but key words can only be entered by typing with a physical keyboard.

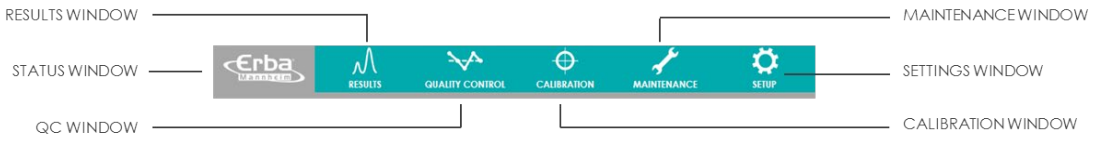
7.2.6. Maximize

The Maximize button will restore the full view of the maintenance window that has been moved away from the view. During some maintenance (cleaning) procedures the window can be moved away to access the other menus of the software. Then the icon display in the bottom menu.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
MAXIMIZE		ABSENT	This button is only visible when a maintenance procedure has been minimized, click on it to restore the normal view of the maintenance procedure window	button

7.3. Top Bar

Once logged in, the Top Bar becomes visible. Apart from the Login screen, the Top Bar is visible in all of the instrument's other Windows and provides a method of moving between these different windows.



7.3.1. Status button

The Status button opens Status window, which displays: the number of samples / tests remaining on the Colum to be monitored; and allows the ordering of sample runs; the review of information for tubes registered on the system; current system parameters and other useful information. It is the main Window used for controlling the instrument.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Status Window			Where samples can be programmed and loaded on the rack and general status can be seen (run, sample tubes, reagent supply level, sensors status, etc.)	Button

7.3.2. Results button

The Results button opens the Results Window which allows for the detailed review of data collected by the system with regards to patient sample, Control and Calibrator sample results. This includes chromatography and calculated results, with relevant information on when the sample was run, by whom and whether any errors were detected during analysis.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Results window	RESULTS	RESULTS	Calls for results screen including the chromatographs	Button

7.3.3. Quality Control button

The Quality Control button opens the Quality control window which allows Control Lots to be added to the system and monitored via QC tables with integrated error flagging and Levey-Jennings charts with monitoring statistics.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Quality control			Calls for all commands and windows linked with Quality Controls (QC), from request, creating new lots of controls, reviewing quality control results and data, statistical analysis, etc...	Button

7.3.4. Calibration button

The Calibration button opens the Calibration window which allows Calibrator Lots to be added and Calibration runs to be ordered. It also provides a method of reviewing Calibrator data and Calibration Curves.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Calibration			Calls for all commands and windows linked with calibrations, from request, creating new lots of calibrators, reviewing calibration results and data	Button

7.3.5. Maintenance button

The Maintenance button opens the Maintenance window which allows a scalable approach to instrument technical service form easy, semi-automated, and automated diagnostics, through to technical service level access to the instrument. It is also the place where kit changes are performed.

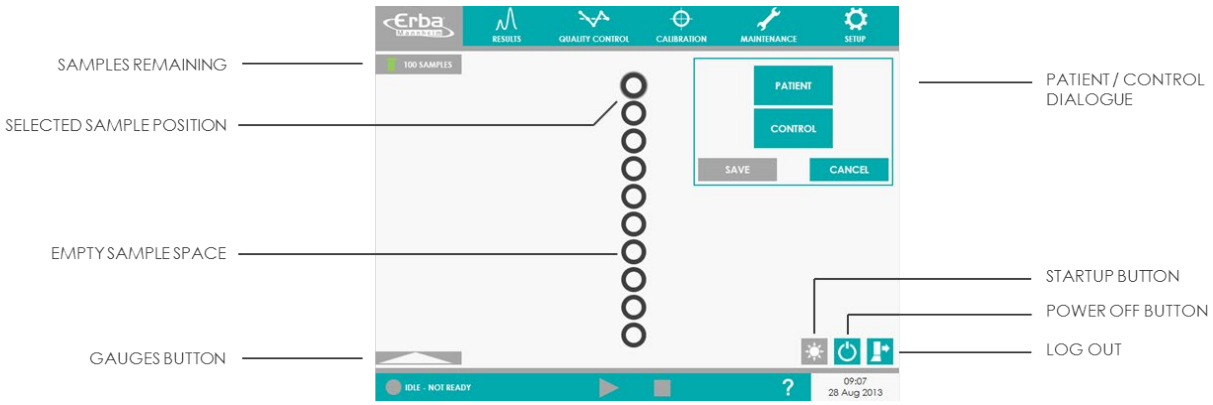
BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Maintenance			Calls for the maintenance protocols from soft maintenance (Prime, Purge, Clean, Report); change of consumables and reagents and service maintenance	button

7.3.6. Setup button

The Setup button opens the Setup windows which allows authorized users to make changes to system settings such as Date / Time formats and Reporting Units and more (see 7.17 Setup for more details).

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Settings			Calls for the parameters of the system	button

7.4. Status Window I: Initial View



7.4.1. Status of reagent supply: Samples/testsremaining

The status of reagent supply area displays the number of samples/tests remaining in the kit / column combination. The column displayed here will turn from green to red when there are fewer than 10 samples remaining.

Note: the initial conditioning of the column and the initial calibrations are not counted down from Column/kit. This leaves 100 tests for controls and patients' tests.

Normally, 1 calibration should be needed per column. 3 calibrations maximum can be performed for each column without deducting them from the column life. From the 4th calibration, each tests performed will be deducted.

DISPLAY	NORMAL STATUS	ALARM STATUS	DESCRIPTION / USE	FORMAT
Column tests left			This status displayed the column in color code and the number of tests left into the column. If the number of tests is still OK, it displays the column in green. If the number of tests is 10 or less than the number of tests defined in settings, then it displays the column in red.	Information field + color coded image

7.4.2. Gauges Button: Operating temperatures

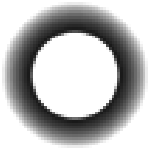
The Gauges Button can be pressed to open the Gauges Panel. This panel displays information on the current system of instrument's temperatures. If there any information in these panels are out of range, this panel will automatically expand. See section 7.6.2 for further details.

They are masked when the temperatures are within specifications:

- Ambient temperature between 17 and 32°C
- Incubator temperature 60°C +/- 1°C
- Column temperature +/- 1°C from the instrument sensor specific target (located between 24.5 and 29°C on display)

7.4.3. Rack tubes information

The rack tubes positions are color coded and a number of related information are displayed on and around the tube area.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
SAMPLE NATURE				
	Empty position		Clicking on an empty position	Popup window opens allowing the choice between a sample or a control (see details in sample / control definition section)
	Selected position		Click on any position (whether defined or empty)	halo of matching color of the color coded tube will surround the tube
	Low calibrator	Calibrators are automatically positioned on the tray first positions from requesting a	Click on a calibrator defined position	The summary of the order is displaying on the right part of the window. It contains: Tube#, Lot Number, Tube

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
	High calibrator	calibration procedure from the calibration window		Type, Sample Type Assay, # of repeats
	Low control	Controls can be automatically positioned on the tray immediately after the calibrators positions from requesting a calibration procedure from the calibration window They can also be positioned from the status window on an empty position	Click on a control defined position	The summary of the order is displaying on the right part of the window. It contains: Tube#, Lot Number, Tube Type, Sample Type Assay, # of repeats
	High control			
	Sample	Samples are positioned from the status window on an empty position, or automatically identified by internal barcode reader. Possible positions 1-10	Click on a sample defined position	The summary of the order is displaying on the right part of the window. It contains: Tube#, Sample ID, Tube Type, Sample Type, Assay, # of repeats
	Cleaner and Rinse	Cleaner is positioned exclusively on position 10 of the rack. Rinse is positioned exclusively on position 9 of	Click on a Cleaner or Rinse position, button	it displays the product type as a grey selected The Save button is inactive, only the Cancel button is active. To remove, click on None and Save

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
	Or unknown position	the rack. The 2 products are needed onboard for the enzymatic cleaning procedure When rack is inserted, the barcode reader reads all positions, if positions are not identified with barcodes the unidentified positions will be marked as orange with the flag unknown 	A message will popup: Empty / unknown positions (X). Automatic start in 00:30. Start/No *(and counting down in seconds)* User can ignore, force to start or press No and amend the unknown position(s).	Answer Start will force immediate start Not answering will start after the 30 sec count down is over Answer No will allow identification of unknown position(s) then press Play  again to start the run
	Number of tests programmed left to be performed	This number is updating in real time and counting down the number of tests still be to be performed.	1. Request 2 repeats of an assay 2. when the system runs tests	1. The tube has a 2 displayed in its center 2. the number will count down as the tests are run
	Sample completed		When requested a number of repeats, tests run	When the last replicate is completed, the number 1 becomes the check mark to indicate the sample

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
				is fully completed
SAMPLE TYPES				
	Whole blood sample	As defined in the sample definition	When requesting a whole blood sample from the sample definition window	The square to the right of the tube position is dark red
	Predilute sample	As defined in the sample definition	When requesting a lysate sample from the sample definition window	The square to the right of the tube position is light red
TUBE TYPES				
	Regular primary	13x75mm	From selection of Regular primary from the sample definition popup window	The “Regular primary” icon displays to the left of the sample position
	Tall primary	13x100mm	From selection of Tall primary from the sample definition popup window	The “Tall primary” icon displays to the left of the sample position
	Sample vial	Eppendorf 1.5ml type As well as microcapillary primary tubes from brands other than Sarstedt Microvette®	From selection of sample vial from the sample definition popup window.	The “Sample vial” icon displays to the left of the sample position

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
		This tube type is positioned into a specific bottom magnetic adaptor on the sample tray.		
	Microcapillary	These are microcapillary primary tubes of Microvette® Sarstedt type exclusively This tube type is positioned into a specific top magnetic adaptor on the sample tray.	From selection of microcapillary from the sample definition popup window.	The “microcapillary” icon displays to the left of the sample position
STATUS				
	Preparing (incubation cycle)		When a sample is aspirated to the end of the incubation cycle	it displays a double orange arrow
	Testing (measurement cycle)		When a sample is being dispensed from the incubator to the loop and to the column and measured	it displays a double blue arrow until result calculation
	Preparing and testing	This case is only possible if more than 1 assay or more than 1 repetition for the same sample is requested	If 2 repeats, 1 is in incubation and the other in measuring phase	it displays a double blue & orange arrow (then switch to a full blue double arrow icon until result calculation of the last replicate

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
	Warning	A possible conflict between definition and detection Or a flag / alert raised for the sample testing	Define a sample in position 6, then load rack without position 6 tube presence Or if loading with barcode, any empty position will be marked as unknown	A warning comes in front of position 6 because of conflict between manual definition and auto position confirmation
	Mismatch	A possible conflict between definition and detection	Define a sample ID in a position, then load the rack with another barcoded sample will trigger this flag	The mismatch flag lists the 2 different sample ID, the user can chose to use the automated barcoded ID or the manual sample ID The manual ID selection will be written in the logs for traceability.

7.4.4. Patient / Control / Cleaners dialogue

The Patient / Control / Cleaners dialogue appears when an Empty Sample Space Icon is pressed. This dialogue prompts the user to present this sample as a Patient, Control sample or cleaners. Selecting either of these options enables the Save button. Cleaner and Rinse are position specific, Cleaner in Position 10 and Rinse in position 9; these are positioned to or when requesting an enzymatic cleaning procedure (to be performed daily).

Pressing Save will validate the position for Rinse or cleaner and will bring up the Sample or control Information Entry dialogue for these 2 latter types (see sections 7.5.3 for details on this dialogue). Only once sufficient information has been entered, will the system allow the sample to be saved and then run.

Figure 15: Patient/Control/Cleaners dialogue: For Pos. 9, for pos. 10, for Pos. 1 to 8

7.4.5. Startup / Standby Button

The Startup / Standby button (a Sun symbol for Startup / a moon symbol for Standby) will depend on the current state of the instrument. If the instrument is 'IDLE, NOT READY' a Startup must be run before samples can be run on the system. Once a Startup has been completed, the Sun symbol turns into the Moon symbol, Standby. A Standby will automatically run after a period of inactivity, turning off the incubator, the LED (Light source) and some pumps. It can be forced by pushing the Moon button directly.

The system will still be IDLE with status STANDBY. To resume testing, just prepare the run and press play, the system will automatically restore power to the needed modules and monitor gauges to be within range before starting testing operations.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
START UP			This button is used to request the instrument startup cycle. Once pressed it first asks a question: "do you wish to start a new workday?" Answering No will continue working with the same memories of sample ID, data, etc. Answering Yes will archive the data and reset the Sample ID, allowing the reuse of the same sample IDs for different samples/patients. The button then toggles to its inactive status. Once the startup cycle is completed it then toggles to the active Standby button	Toggle button

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
Standby (analyser)			This button is used to request the instrument Standby cycle. In the process it gives the user the opportunity to request a daily cleaning procedure before going to Standby mode. Once pressed: 1. the button toggles to its inactive status 2. it opens a dialog box with 3 options: • "Comprehensive Clean and Standby" *to perform the cleaning procedure and automatically going to the standby mode afterwards* • "Standby" *to go directly to the standby mode thus not performing the cleaning procedure* • "Cancel" *which will not perform any operation* 3. Once the Standby cycle is completed, it then toggles to the active Startup button. It is also inactive during the instrument status BUSY	Toggle button

7.4.6. Log Out Button

The Log Out Button logs the current user out and allows a new user to log on via the Login Screen.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
LOGOUT (change user)			This button is used to logout or to switch user. Pressing this button leads to the Login screen Active only in Not ready / ready / standby modes Note: After user switch, the previous status will remain unchanged Ready	Button

			/Ready	

7.4.7. The Power off Button

The Power off Button allows the user to power off the PC, closing Windows properly. Once the screen is black, then the Switch button can be placed on the O position.

BUTTON	DEFAULT / ACTIVE STATUS	SELECTED / INACTIVE STATUS	DESCRIPTION / USE	FORMAT
POWER OFF (computer)			This button is used to request a computer power off. Upon clicking on this button: A. If the system is in Ready or standby status: 4 options will be proposed: • "Comprehensive Clean and Power Off", *to perform the cleaning procedure and automatically power off the computer afterwards* • "Clean and Power Off", *to perform the clean and Power off procedure.* • "Power Off" *to power off directly thus not performing the cleaning procedure* • "Cancel" *which will not perform any operation* B. If the system is in Not Ready status: A confirmation question will be raised. If confirmed an instrument stop will first be executed, then the computer will power OFF. Once the computer is powered off, the user should then switch OFF the instrument by placing the switch button to the O position.	Button

			The button is inactive when the system is BUSY	

7.5. Status Window Part II: Data Entry

Pressing on any of the Empty Sample Space icons, calls up a dialogue which allows the type of sample to be selected. This is known as the Patient / Control dialogue (see section 7.4.4 for details). Selecting either Patient or Control and pressing save inside the Patient / Control dialogue displays the Sample Data Entry dialogue.

7.5.1. Patient Data Entry

7.5.1.1. Sample Programming

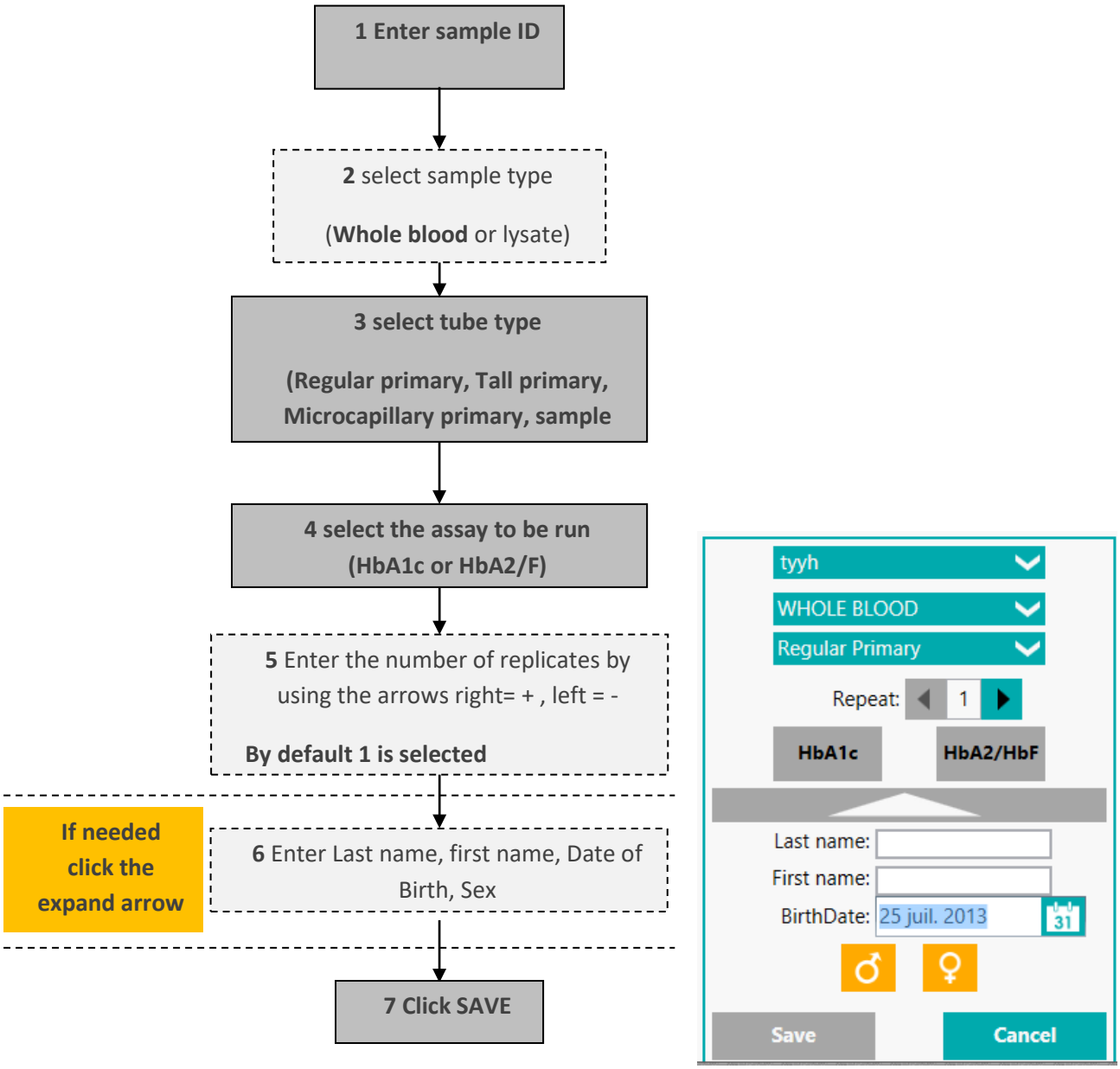
On the right side of the rack display the summary of sample programming for the selected sample is displayed, showing the following information

For a patient, the information displayed are:

- Tube#
- Sample ID
- Tube Type
- Sample Type (Whole blood or lysate)
- Assay
- Number of repeats

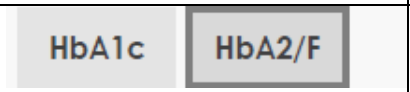
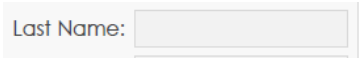
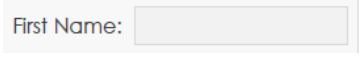
The Sample ID must be unique within a “workday”. To reset the Sample ID and be able to use the same sample ID for other samples, a new Startup must be performed (Refer to 4.6 Startup for more information).

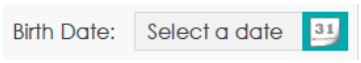
7.5.1.2. Sample Definition Popup Window



7.5.1.3. Details of the Sample Definition Window

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
Sample ID		Placing the cursor in the name field will open the virtual keyboard. A new sample ID must be typed (maximum 20 characters). A sample Id is unique within a "workday", but the same sample ID can be reused every new workday. It is also possible to recall a sample ID from the list in the combo box. (the sample ID display within the same workday) To generate a new workday, the user needs to perform a new Startup	Editable Combo box
Sample type	 Options are : • Whole blood • Lysate	Select from the drop down list. By default **Whole blood** will be selected Click on the arrow to open the list and move the finger (pointer) to the desired option to select it	Combo box
Tube type	 List of available choices: • Regular primary, • Tall primary, • microcapillary primary, • sample vial	Select from the drop down list. By default **Regular Primary** will be selected Clicking on the combo box will open the list Move the finger (pointer) to the desired option to select it	Combo box
Assay choice		Select the assay(s) HbA1c or HbA2/F	Selectable Button

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
	 Individually  	By default **the assay** selected as default in Setup HbA1c or HbF/HbA2 will be selected The selection is visible by the surrounding line Clicking on one option will select it Selecting the other option will deselect the first one. Clicking again on the selection will unselect it It is possible to select only 1 type of assay at a time. Of the other assay is also needed it can be added once the first assay is completed.	
Repeat		Use the arrows to the right to increase the number of repeats Use the arrow to the left to decrease the number of repeats By default the number of repeat set in Setup / Assay is selected	
Last name		Click in the Last name field The keyboard will automatically open Type any name (maximum 35 alphabetic characters) and press enter to see it displayed in the field the keyboard closes automatically and the cursor stays in the field after the last character Re-click to reopen the keyboard	Free Text
First name		Click in the first name field The keyboard will automatically	Free Text

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		open Type any name (maximum 35 alphabetic characters) and press enter to see it displayed in the field the keyboard closes automatically and the cursor stays in the field after the last character Re-click to reopen the keyboard	
Birthdate		Click on the field to open the keyboard: the date can be typed in date format (future dates are disabled and not accepted) Click on the calendar icon to open the calendar options See details in the calendar section	Free text date format & Calendar combo
Sex	 = MALE  = FEMALE	The sex is selected by clicking on the corresponding symbol Once selected the button is surrounded by a grey line Re-click on a selected button will deselect it	Selectable buttons
Save		The save button is inactive until all needed information are filled Click on the save button to save the request	Button
cancel		Click Cancel to get out of the window without changing r saving any information	button

7.5.2. Control Data Entry

7.5.2.1. Summary of Control Data Entry

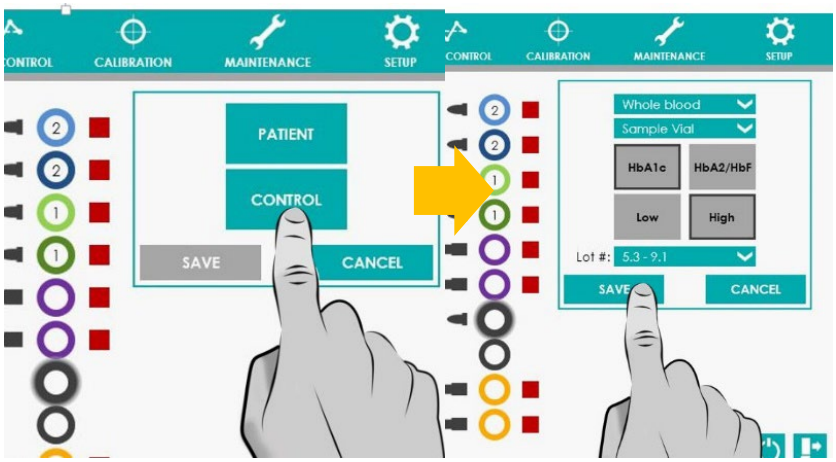
For a control or calibrator the displays are:

- Tube position
- Lot number
- Tube type
- Sample type (Whole blood or lysate)
- Assay
- Number of repeats

7.5.2.2. Control definition popup window

The Control Data Entry dialogue is only composed of 1-part.

Upon pressing on an empty position, click on Control button, then save. The control entry dialogue opens, requiring the selection of the: analyte(s) (HbA1c or HbA2/hbF), the control level (Low of High), the lot number of the control, then save.



The position will be displayed in green (Light green for the low levels and

1 select the sample type
(Whole blood or lysate;
default is whole blood)

2 select tube type

(Regular primary, Tall primary, Microcapillary, **sample vial**;
Default is sample vial)

3 select the assay to be run

(HbA1c or HbA2/F; *default test is as selected in setup)*

4 select the control level

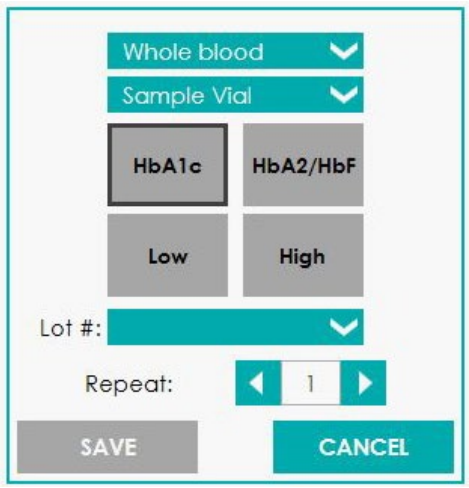
(Low or High)

5 select the lot number from the combo box

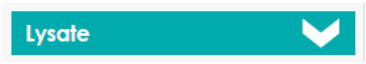
6 select the number of repeats

(*Default is 1)*

6 Click on Save



7.5.2.3. Details of the Control Definition Window

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
Sample type	 Options are : • Whole blood • Lysate	Select from the drop down list. *By default **Whole blood** will be selected* Click on the arrow to open the list and move the finger (pointer) to the desired option to select it	Combo box
Tube type	 List of available choices: • Regular primary, • Tall primary, • microcapillary primary, • sample vial	Select from the drop down list. *By default **Sample vial** will be selected* Clicking on the combo box will open the list Move the finger (pointer) to the desired option to select it	Combo box
Assay choice	 Individually  	Select the assay between HbA1c and HbA2/F *The default **method** will be selected* Clicking on one option will select it, the selection is visible by the surrounding line Only 1 selection within the assay choice is possible Click on the other choice will select the other choice and unselect the current one Clicking again on the selection will unselect it	Selectable button
Control level choice		Select the control level between Low and High *No default selection is made.* Clicking on one option will select it, the selection is visible by the surrounding line Only 1 selection within the assay choice is possible Click on the other choice will select the other choice and unselect the current one Clicking again on the selection will unselect it	
Lot #		Click on the Lot # button to open the list of existing lot numbers. Only the defined lots appear	Combo box

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		Only the non-expired lots appear It is not possible to create a lot number here Until the lot number is entered the save button is not active	
Repeat		*The default **number of repeats** will be selected as per setup* Use the arrows to the right to increase the number of repeats Use the arrow to the left to decrease the number of repeats	
Save		The save button is inactive until all needed information are filled Click on the active save button to save the request	Button
Cancel		Click Cancel to get out of the window without changing or saving any information	button

7.5.3. Rinse and Cleaner Entry

When the user is willing to run a cleaning procedure at the end of the sample run, the Rinse and Cleaner can be positioned on the rack in respective positions 9 and 10. The 2 products need to be both present for the cleaning procedure to be automatically initiated at the end of the run.

- The Rinse needs to be placed in a specific “10 ml” tube provided in the Cleaner kit, as well as in the Hb-Vario kit: Fill the tube with at least 10 ml of Rinse, place in position 9
- The Cleaner can be placed in a 5ml tube, and contain at least the requested 4ml of Cleaner, place it in position 10.

To program them onboard, follow the steps described in 7.5.3.1 Rinse Entry and 7.5.3.2 Cleaner Entry

Warning: The order of positioning of the 2 different cleaning solutions Cleaner and Rinse is extremely important and cannot be reversed, or **the column will be damaged**. Insure that Cleaner is physically positioned on the rack in position 10, and the Rinse in position 9.

When requested by positioning the Rinse and Cleaner on the rackthe process of the cleaning procedure is as follows:

1. The sample run will be completed, and the instrument will be placed in Pause.
2. The instrument will prompt for the cleaning procedure with an automatic count-down of 30 minutes.

The user can:

- Press cancel and the procedure will be cancelled.
- Press continue and the procedure will start
- No action from the user within the 30 minutes count down and the procedure will be automatically started.

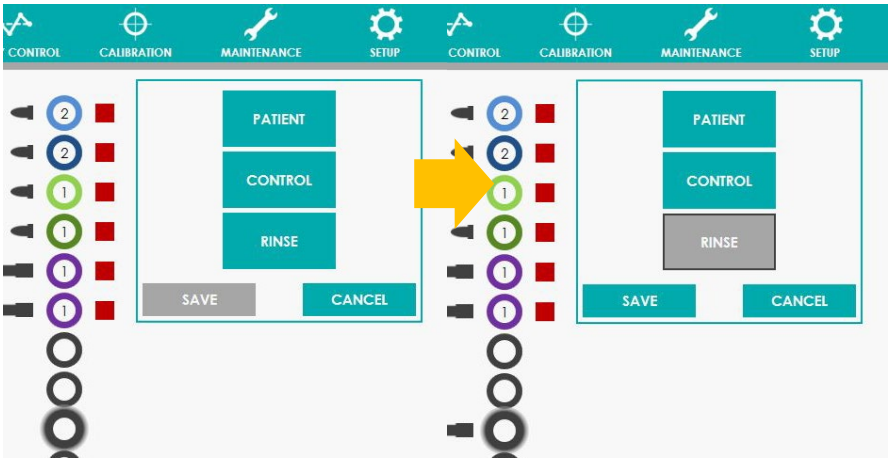
The procedure lasts a little over 20 minutes:

1. First the system awaits to reach a temperature of 60°C(+/-1°C) in the incubator
2. Then it aspirates the Cleaner,
3. Proceeds with the cleaning of the loop, incubator and sampler, leaving in contact for 5 minutes regulated at 60°C.
4. Rinses the low pressure system, the sampler, and incubator at 60°C
5. Then rinses the loop and low pressure system, the sampler and incubator at 60°C
6. Then repeats steps 4 and 5.

Warning: Make sure that the Cleaner and Rinse solutions are not placed in barcoded tubes. Barcoded tubes will be interpreted as samples to be run. Normal aspirations and tests would then be performed from these tubes, inserting enzymatic cleaner into the column, **thus damaging it and making it improper for further testing.**

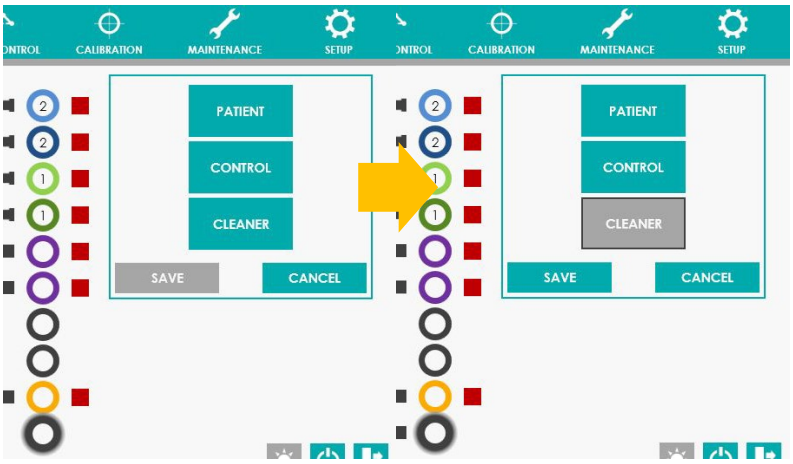
7.5.3.1. Rinse Entry

To program the Rinse onboard, click on the empty position 9, select Rinse and Save

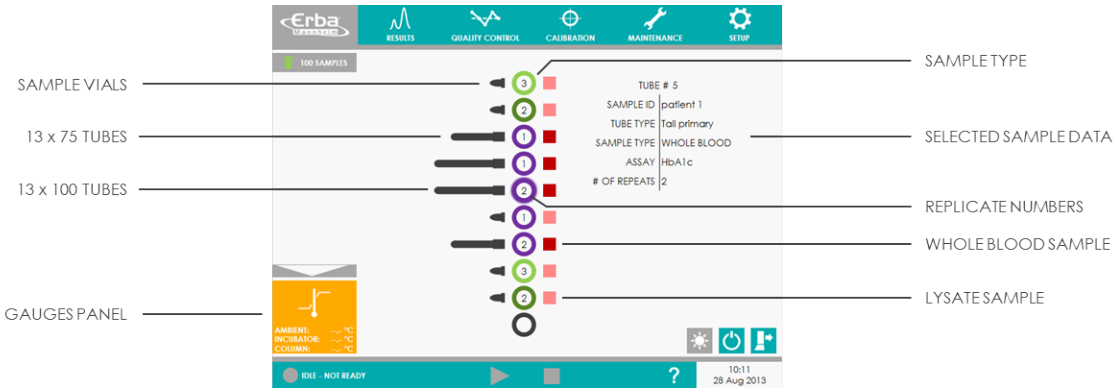


7.5.3.2. Cleaner Entry

To program the Cleaner onboard, click on the empty position 10, select Cleaner and Save



7.6. Status Window Part III: Reviewing Data



7.6.1. Tube Type Icons

As shown above the Hb-Vario will accept different Tube Types and each type is represented graphically in the Status Screen. The Hb-Vario will accept the following types of tube / sample receptacle:

- **Sample Vials:** 1.5 ml Sample Vials
- **Regular Primary:** 13 x 75 mm Sample Tubes (with caps removed)
- **Tall Primary:** 13 x 100 mm Sample Tubes (with caps removed)
- **Microcapillary:** Microcapillary Vials (with caps removed). These refer to the Sarstedt microvette, 100µl, 200µl or 500µl. the bottom of these microcapillaries are higher than the adaptor support, therefore they are placed in an adaptor with the high magnet position.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
TUBE TYPES				
	Regular primary	13x75mm	From selection of Regular primary from the sample definition popup window	The “Regular primary” icon displays to the left of the sample position
	Tall primary	13x100mm	From selection of Tall primary from the sample definition popup window	The “Tall primary” icon displays to the left of the sample position
	Sample vial	Eppendorf 1.5ml type This tube type is positioned into a specific magnetic adaptor (low position) on the sample tray.	From selection of sample vial from the sample definition popup window.	The “Sample vial” icon displays to the left of the sample position
	Microcapillary	These are microcapillary primary tubes This tube type is positioned into a specific magnetic adaptor (high position) on the sample tray.	From selection of microcapillary from the sample definition popup window.	The “microcapillary” icon displays to the left of the sample position

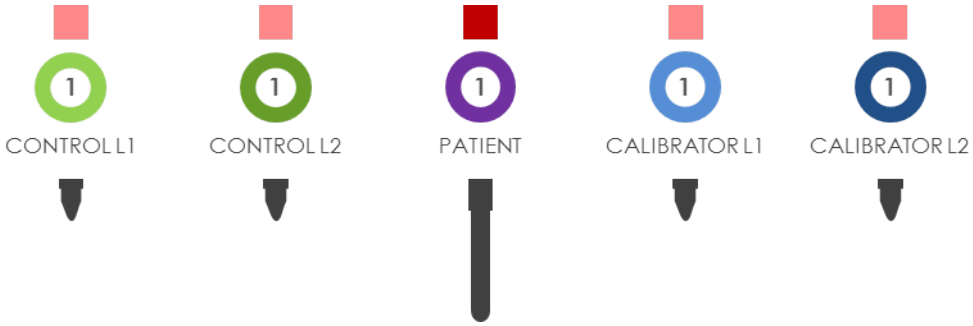
7.6.2. Gauges Panel

Shows the Gauges Panel when expanded. Pressing the arrow to open the panel, pressing at the top of this panel closes the panel again.

They are masked when the temperatures are within specifications:

- Ambient temperature between 17 and 32°C
- Incubator temperature 60°C +/- 1°C
- Column temperature located between 24.5 and 29°C on display

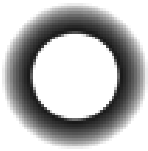
7.6.3. Sample Type Colors



Different Sample Types have different Sample Position colors

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
SAMPLE NATURE				
	Empty position		Clicking on an empty position	Popup window opens allowing the choice between a sample or a control (see details in sample / control definition section)

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
	Low calibrator	Calibrators are automatically positioned on the tray first positions from requesting a calibration procedure from the calibration window	Click on a calibrator defined position	The summary of the order is displaying on the right part of the window. It contains: Tube#, Lot Number, Tube Type, Sample Type Assay, # of repeats
	High calibrator			
	Low control	Controls can be automatically positioned on the tray immediately after the calibrators positions from requesting a calibration procedure from the calibration window They can also be positioned from the status window on an empty position	Click on a control defined position	The summary of the order is displaying on the right part of the window. It contains: Tube#, Lot Number, Tube Type, Sample Type Assay, # of repeats
	High control			
	Sample	Samples are positioned from the status window on an empty position, or automatically identified by internal	Click on a sample defined position	The summary of the order is displaying on the right part of the window. It contains: Tube#, Sample ID, Tube Type, Sample Type, Assay, # of repeats

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
		barcode reader. Possible positions 1-10		
	Cleaner and Rinse	Cleaner is positioned exclusively on position 10 of the rack. Rinse is positioned exclusively on position 9 of the rack. The 2 products are needed onboard for the enzymatic cleaning procedure	Click on a cleaner or Rinse position, button	it displays the product type as a grey selected The Save button is inactive, only the Cancel button is active. To remove, click on None and Save
	Selected position		Click on any position (whether defined or empty)	halo of matching color of the color coded tube will surround the tube

7.6.4. Selected Sample Data

When an occupied Sample Space Icon is selected the data entered for this sample is displayed alongside the rack representation. Pressing any part of this area of the screen will allow the data for that sample to be altered as long as the system is not currently preparing or running the relevant sample. For this, the user must first confirm the sample nature (Patient, Control, Rinse *, Cleaner**, or none).

If the same as current nature is confirmed, the previously recorded information will be displayed. Information can be added or modified.

* on appropriate position (9)
** on appropriate position (10)

7.6.5. Replicate Numbers

The number in the center of each Sample Space Icon denotes the number of times the instrument will run that test for that tube. This can be changed by pressing on the desired sample and then pressing on the Selected Sample Data area. Possible number of replicates: 1 to 10

Once all tests to be performed are complete and reported, a check mark is displayed in the center of the tube.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
	Number of tests programmed left to be performed	This number is updating in real time and counting down the number of tests still to be performed.	1. Request 2 repeats of an assay 2. When the system runs tests	1. The tube has a 2 displayed in its center 2. The number will count down as the tests are run
	Sample completed		When requested a number of repeats, tests run	When the last replicate is completed, the number 1 becomes the check mark to indicate the sample is fully completed

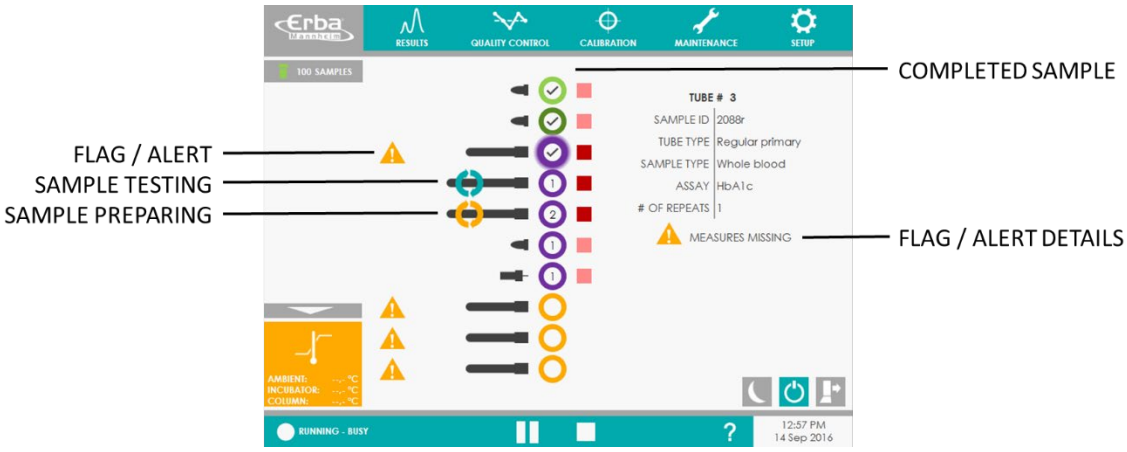
7.6.6. Lysate and Whole Blood Sample Identifiers

Dark red squares indicate that this is a whole blood sample and pink squares indicate that this is a lysate (pre-dilute) sample.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
SAMPLE TYPES				
	Whole blood sample	As defined in the sample definition	When requesting a whole blood sample from the sample definition window	The square to the right of the tube position is dark red
	Predilute sample	As defined in the sample definition	When requesting a lysate sample	The square to the right of the tube position is light red

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
			from the sample definition window	

7.7. Status Window Part IV: Running Samples



7.7.1. Flag / Alert Icon

On the status window different Flag / Alert icons could be displayed to warn the user of different important pieces of information.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION(S)	RESULT
STATUS				
	information	On a rack position before the run it means that no tube was detected by the barcode reader A message numbering the positions will display with an auto start countdown of 30 sec	Do not answer the Yes/No question Answer Yes to confirm that the flags positions are indeed empty Answer No	The system will go after the 30sec elapsed time The run will start immediately The rack will be ejected to be able to

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION(S)	RESULT
				read it again
	information	On a sample after it was run it is an alert raised for the sample testing (result or reaction flag)	Review the alert by clicking on the flag	It opens the summary window to give the alert reason(s)
	Warning	On a rack position before the run, it means that there is a mismatch between the barcoded tube identified by the barcode reader and the sample manually identified by the test request information	Click on the alert icon to choose the ID to be used from: →Barcode reader identification →Manual entry	The system will use the selected ID

7.7.2. Sample Preparation Icon

As soon as the system moves to aspirate sample from a tube position, that sample is being prepared and the Sample Preparation, a rotating yellow / orange arrow icon, will be displayed over that sample's Tube Type icon.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
STATUS				
	Preparing (incubation cycle)		When a sample is aspirated to the end of the Incubation cycle	It displays a double orange arrow

7.7.3. Sample Analysis Icon

As soon as the system injects the sample onto the column, that sample is being analyzed and the Sample Analysis, a rotating blue / teal arrow icon, will be displayed over that sample's Tube Type icon.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
STATUS				
	Testing (measurement cycle)		When a sample is being dispensed from the incubator to the loop and to the column and measured	It displays a double blue arrow until result calculation

7.7.4. Sample Preparation and Analysis Icon

If a sample is scheduled to run more than a single replicate then at some point in the run the instrument will be preparing and analyzing this same at the same time. When this is the case, the system will display a half blue / half yellow rotating arrow over that sample's Tube Type icon:

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
	Preparing and testing	This case is only possible if more than 1 assay or more than 1 repetition for the same sample is requested	If 2 repeats, 1 is in incubation and the other in measuring phase	it displays a double blue & orange arrow (then switch to a full blue double arrow icon until result calculation of the last replicate

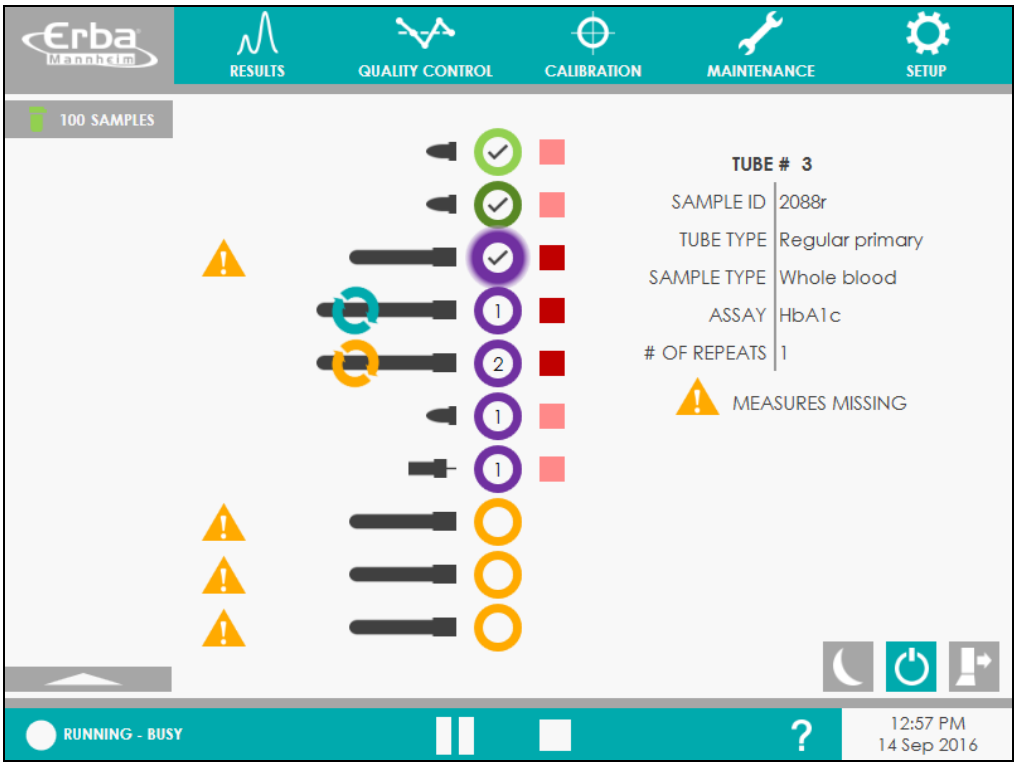
7.7.5. Completed Sample Icon

Once the designated number of replicates for any sample has been run, the system will place a tick in the center of the Sample Space icon for that sample.

GRAPHIC	MEANING	ADDITIONAL INFO	ACTION	RESULT
✓	Sample completed		When requested a number of repeats, tests run	When the last replicate is completed, the number 1 becomes the check mark to indicate the sample is fully completed

7.7.6. Flag / Alert Details

If a sample has a flag or alert raised against it, pressing the sample's Sample Space Icon will display the sample data for this sample as well as the flag or alert raised against this sample. The flag or alert will be noted beneath the sample data area.



7.8. Results Window: Patient Samples

The Results Window allows the view and review of sample, Control and Calibrator data:

7.8.1. Patient Information

Patient information, entered when the sample is loaded onto the system, is displayed here.

7.8.2. Chromatogram (Trace)

When displaying old sample data the chromatography is displayed here. Chromatogram will be displayed live when viewing the sample which is currently running.

7.8.3. Flags / Alerts

Any Flags / Alerts associated with this sample data will be displayed in this box at the bottom of the page. If the sample is being displayed live, these Flags / Alerts will only display once the sample has been completely analyzed.

7.8.4. Previous Button

Pressing the Previous Button moves backwards through the sample database sequentially, allowing each sample which has been run, to be reviewed.

7.8.5. Zoom Button

The Zoom Button allows entire chromatogram to be view. Normally, a zoomed in version of the chromatograph is show so that a more convenient view of HbA1c, HbF and HbA2 can be provided.

7.8.6. Results Table

The Results Table displays all peak data for the sample being viewed. Calibrated % results are displayed for the HbA1c, HbF and HbA2 peaks where possible.

Note: Calibrated results can only be displayed for the calibrated analyze run, i.e. if HbA1c has been run then calibrated results for HbA2 and HbF cannot be provided.

7.8.7. Results in eAG

The estimated average glucose eAG can derived from the HbA1c calibrated result if the setup for this is set 'ON' in the Settings Window. See 7.17.5 Setup, HbA1c for more information.

7.8.8. Results in 2nd unit

Like the eAG, a 2nd unit for the HbA1c expression can be optionally activated from the Setup, HbA1c window. For example a laboratory can choose to work with IFCC unit but display results in IFCC and NGSP on the same report.

7.8.9. Scroll Flags / Alerts

Should there be too many Flags / Alerts attached to a single sample for them all to fit in the window provided, scroll buttons will appear in the right hand side of the box.

7.8.10. Next Button

Pressing the Next Button moves forwards through the sample database sequentially, allowing each sample which has been run, to be reviewed. If a sample is currently being analyzed, it will be the last sample in the database.

7.8.11. Print Result Button

The Print Result Button allows the sample currently on-screen to be printed.

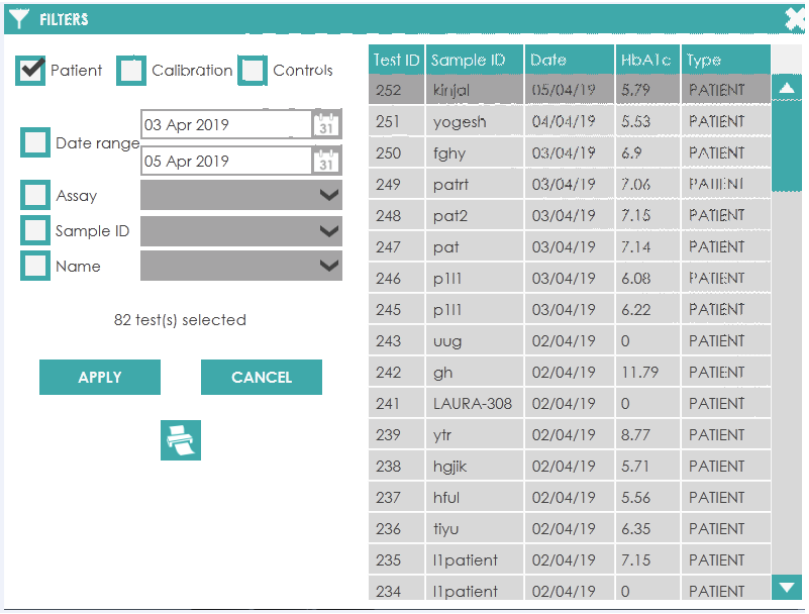
7.8.12. Send to LIS Button

The Send to LIS Button allows the sample currently on-screen to be sent to the connected LIS.

7.8.13. Filter results

The results displayed in the results window are by default only the ones for the last operation day. Older results can still be retrieved by filtering them. Filtering can be done for

- Patient, Controls or calibrators (which includes actual calibrators, primers and blanks).
- Date range
- Assay
- Sample ID
- Name



7.8.14. Print Result Button

The Print Result Button  allows to print result summary as shown below

PRINTED: 12 Apr 2019 3:55 PM

Erba Mannheim

Hb-Vario

RESULT SUMMARY

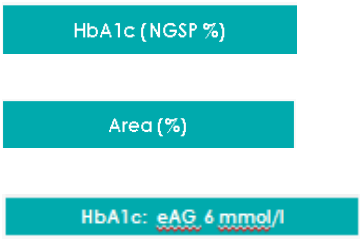
FILTERS Type : Patient
Date range : 01-01-19 - 01-01-19

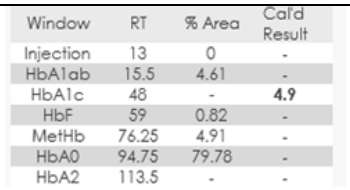
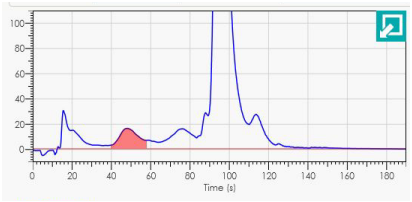
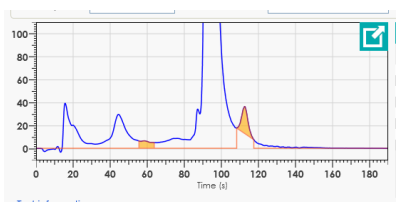
S/N: ERROR **SOFTWARE:** Debug

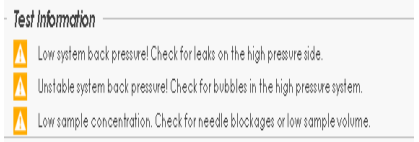
Test ID	Sample ID	Date	HbA1c	Type
15	34Ac	01/01/2019	5.51	PATIENT
14	def	01/01/2019	5.51	PATIENT
13	def	01/01/2019	5.46	PATIENT
12	def	01/01/2019	5.75	PATIENT
11	def	01/01/2019	5.74	PATIENT
10	def	01/01/2019	5.72	PATIENT
9	def	01/01/2019	5.77	PATIENT
8	def	01/01/2019	5.61	PATIENT
7	def	01/01/2019	5.66	PATIENT
6	def	01/01/2019	5.7	PATIENT
5	def	01/01/2019	5.35	PATIENT
4	ABC	01/01/2019	5.6	PATIENT
3	ABC	01/01/2019	5.66	PATIENT
2	12345	01/01/2019	6.1	PATIENT
1	12345	01/01/2019	6.04	PATIENT

7.8.15. Details of the result window (for sample display):

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
Sample information area			
Test #		The Test# is a unique number assigned to the test in the database This field cannot be changed by the user, cannot be click or accessed. This field will always be filled with information when the result window displays a trace It appears as soon as the sample entered in the column during measurements	Information field
Sample ID		Information displayed linked to the trace and results This information is found from the test programming done from Status window, this ID is unique within a workday only This field will always be filled with information for any sample trace displayed	Information field
Date / Time		Information displayed linked to the trace and results The date and time will first display “running” during the measurement and upon result calculation the date and time of calculation This field will always be filled with information for any sample trace displayed	Information field
Name		Information displayed linked to the trace and results This information is the compilation of the Last name and first name as programmed in the test request (from the Status window, sample programming popup window, extended options)	Information field

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		If no information was entered for the request, this field will stay empty	
Assay		Information displayed linked to the trace and results The assay name is the assay that was run to obtain the results displayed (HbA1C or HbA2/HbF) This field will always be filled with information for any sample trace displayed	Information field
Position		Information displayed linked to the trace and results The position is the position number on the rack when the result is consulted when the rack is still in the analyser. Once the rack is unloaded this information field will be blank	Information field
Result table area			
title		This area is variable depending on the assay run and the unit chosen 1. HbA1c (NGSP%) 2. HbA1c (IFCC %) 3. HbA1c (IFCC mmol/mol) 4. HbA1c (Mono-S %) 5. HbA1c: eAG (unit) 6. Area (%) The 5 first ones are for HbA1c assay The 4 first ones are the HbA1C results with respective units/norms (NGSP or IFCC or Mono-S) The 5thone is a calculation of estimated average glucose based on the HbA1c result. This field only displays if setup (presence and unit) The latter for HbA2/HbF assay, in calibrated % area It indicates the unit for the calibrated result column	Information field
Table content		The results are displayed in 4	table

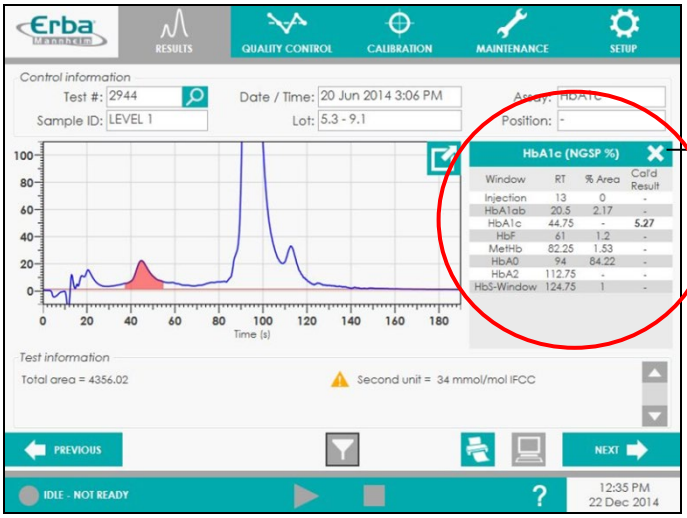
FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
	 Window RT % Area Cal'd Result Injection 13 0 - HbA1ab 15.5 4.61 - HbA1c 48 - 4.9 HbF 59 0.82 - MetHb 76.25 4.91 - HbA0 94.75 79.78 - HbA2 113.5 - - Window RT % Area Cal'd Result Injection 15.5 0 - HbA1ab 21.5 4.37 - HbA1c 46.75 9.06 - HbF 54.25 - 1.62 MetHb 77.5 2.49 - HbA0 90.25 80.58 - HbA2 112 - 1.27 HbS-Window 120.25 0.83 -	columns: Window, where the elements windows are labelled RT for Retention time in sec, where the peak top is found % area, where the % area in function of the element is displayed (for instance HbF is displayed as the % area of all hemoglobin's, where the A1c is expressed as a % of the HbA populations content). Therefore it is possible that the total be greater than 100% The Cal'd results (for calibrated results): Only the calibrated analytes are displayed in this column, so depending on the assay selected: • HbA1c for HbA1c assay • HbF & HbA2 for HbA2/HbF assay The lines of the table are dynamic and created in function of the sample results and peak identification Asterisk can be present to be linked to the message area or calling for professional interpretation	
Trace area			
Trace	 **Figure 16: HbA1C assay chromatogram**  **Figure 17: HbA2 / HbF assay chromoto-**	The trace is the actual chromatogram obtained from elution of the sample in the column A specific area can be select by framing it on the graph, to zoom in Double click on the graph will resume normal zoom display With a mouse wheel it is possible to zoom in axes or graph In the HbA1c assay, the A1c peak is identified by a transparent red	graph

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
	gram	area In the HbA2/HbF assay, the HbF & HbA2 peaks are identified by transparent orange areas	
Fit to view		Allows to display the entire trace	button
Normal display		Returns to the initial zoom display	button
Information area			
Information zone		Under the trace area; the test information area shows details of alarms, flags, messages with little warning logos linked to the selected sample. This zone is equipped of a scrolling bar to move up and down in the list to view all lines It also displays the total area of the sample, the information of whether the assay was run as Whole Blood or Lysate and the 2nd unit if one is selected for the calibrated assay	Information field
Buttons area			
Previous		Allows the user to navigate among the results traces Click on previous to get to the previous trace This button is only active when there are previous result(s) present	button
Next		Allows the user to navigate among the results traces Click on Next to get to the next trace This button is only active when there are more result(s) following the current displayed trace	button
FILTER		This button is used to recall	Button

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		results from database, even previous from the running day and can be searched by: • Patient, Controls or calibrators *(which includes actual calibrators, primers and blanks).* • Date range • Assay • Sample ID • Name	
LIS		This button is used for resending manually the results of the current sample to the LIS	Button
PRINT		Click on this button to print or reprint the result report	button

7.9. Results Window Part II: Control Samples

The Results Window is also capable of displaying Control's. When a Control is reviewed the screen will look as follows:



CONTROL ACCEPTANCE AREA

7.9.1. Rejecting / Accepting a Quality Control result

Pressing 'X' provides a dialogue box which allows a Control to be rejected. Rejected Controls have red Results Table headers and footers:

Controls can also be reintegrated from this window by clicking again on the Check mark ✔ and doing the reverse operation

Rejected Controls are covered later in this manual. See sections 7.11.7 for further details.

7.9.2. Window display for the different types of products

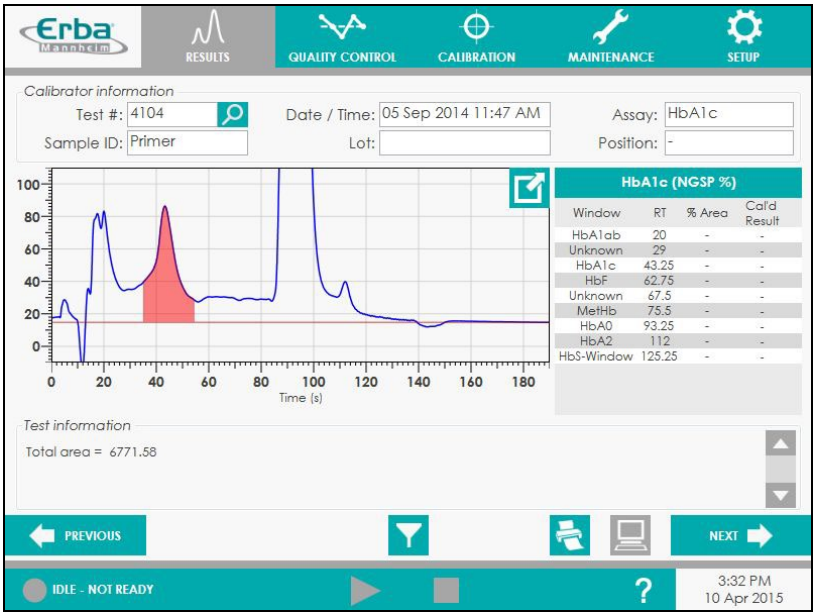


Figure 18: Primer trace (classified under Calibrators)

Figure 19: Blank chromatogram (classified under calibrators)

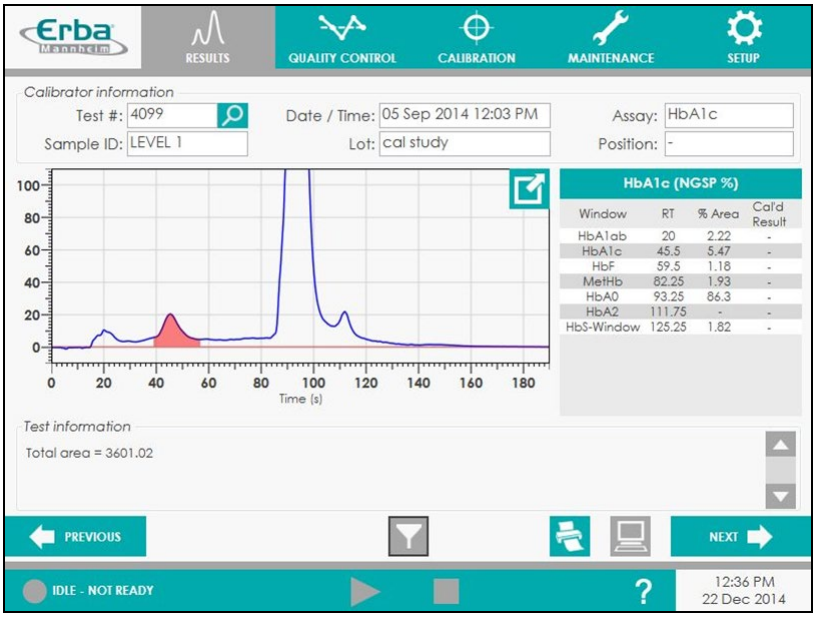


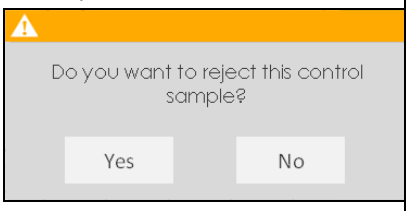
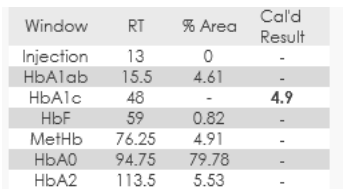
Figure 20: Calibrator chromatogram

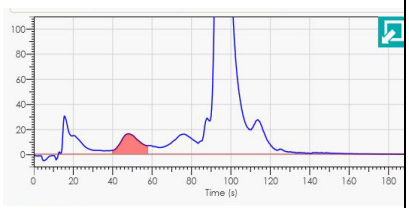
7.9.3. Details of the result window (for Calibrator or Control display)

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
Control (or calibrator) information area			
Test #		The Test# is a unique number assigned to the test in the database This field cannot be changed by the user, cannot be click or	Information field

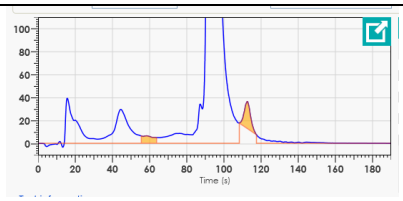
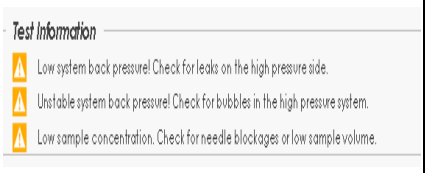
FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		accessed. This field will always be filled with information when the result window displays a trace It appears as soon as the sample entered in the column during measurements	
Sample ID		Information displayed linked to the trace and results This information is found from the test programming done from Status window, this is the coded name of the control or calibrator. It can also say Primer or Blank for those specific procedures linked to a new column conditioning This field will always be filled with information for any control, calibrator, primer or blank trace displayed	Information field
Date / Time		Information displayed linked to the trace and results The date and time corresponds to the end of the assay, when the result was calculated This field will always be filled with information for any sample trace displayed	Information field
Lot		Information displayed linked to the trace and results This information is the lot number of the control or calibrator run (as requested during the demand)	Information field

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		This field is always filled for calibrator or control, but it would be left empty in cases of Primer and Blank	
Assay		Information displayed linked to the trace and results The assay name is the assay that was run to obtain the results displayed (HbA1C or HbA2/HbF) This field will always be filled with information for any sample trace displayed	Information field
Position		Information displayed linked to the trace and results The position is the position number on the rack when the result is consulted when the rack is still in the analyser. Once the rack is unloaded this information field will be blank	Information field
Result table area			
title	 	This area is variable depending on the assay run, the unit chosen, and the status of the control (accepted or rejected) 1. HbA1c (NGSP%) X 2. HbA1c (IFCC %) X 3. HbA1c (IFCC mmol/mol) X 4. HbA1c (Mono-S %) X 5. Area (%) X 6. HbA1c (NGSP%) ✓ 7. HbA1c (IFCC %) ✓ 8. HbA1c (IFCC mmol/mol) ✓ 9. HbA1c (Mono-S %) ✓ 10.Area (%)✓ The 5 first ones are accepted QC, the X means that clicking on the button will reject the QC from	Text / button combined area

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		here (it is also possible to reject QC points from the QC window) The last 5 ones are displayed on Red because they are rejected QC points. Clicking on the teal button will ask a question  Clicking YES will reject the point and the button will toggle to the Red display Clicking NO will have no change. Clicking on the Red button will trigger a question: "Do you want to reintegrate this control?" Clicking YES will change its status to Accepted Clicking NO will have no change effect. The Red button will also display if the QC point has been rejected from the Quality Control menu The 1st, 2nd, 3rd; 4th and 6th, 7th 8th 9th are for HbA1C assay QC results The 5th and 10th are for HbA2/HbF assay, in calibrated ¨% area It indicates the unit for the calibrated result column	
Table content		The results are displayed in 4 columns: Window, where the elements windows are labelled RT for Retention time in sec, where the peak top is found % area, where the % area in	table

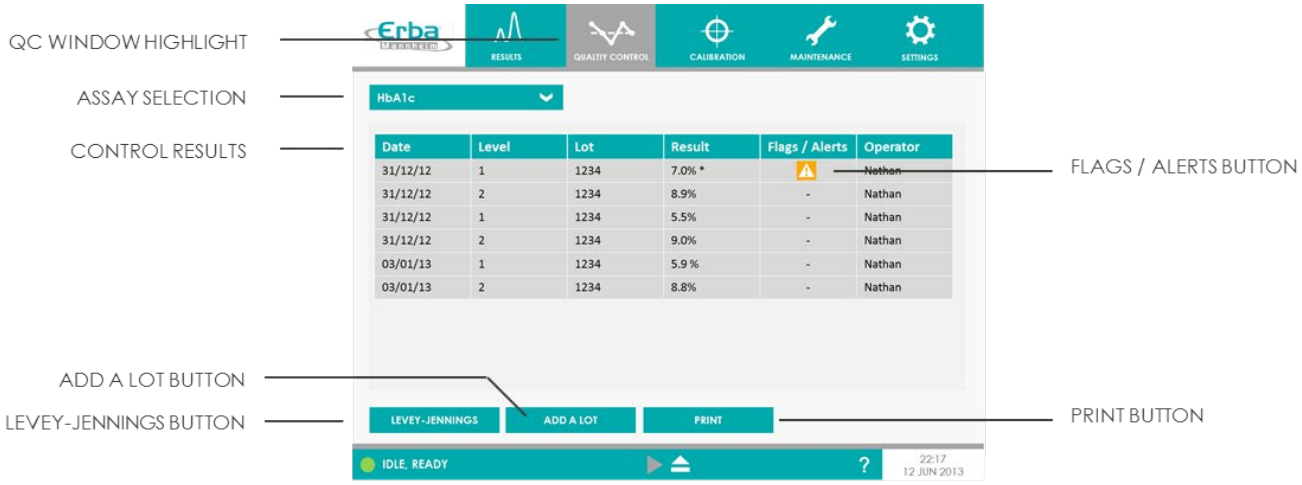
FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		function of the element is displayed (for instance HbF is displayed as the % area of all hemoglobin's, where the A1c is expressed as a % of the HbA populations content). Therefore it is possible that the total be greater than 100% The Cal'd results (for calibrated results): this column is empty for Primer, Blank, calibrators. Only the calibrated analytes are displayed in this column, so depending on the assay selected: • HbA1c for HbA1c assay • HbF & HbA2 for HbA2/HbF assay The lines of the table are dynamic and created in function of the sample results and peak identification Asterisk can be present to be linked to the message area or calling for professional interpretation	
Trace area			
Trace	 **Figure 21: HbA1C assay chromatogram**	The trace is the actual chromatogram obtained from elution of the control and calibrator material in the column Behavior is the same as for sample trace In the HbA1c assay, the A1c peak is identified by a	graph

Window	RT	% Area	Cal'd Result
Injection	15.5	0	-
HbA1ab	21.5	4.37	-
HbA1c	46.75	9.06	-
HbF	54.25	-	**1.62**
MetHb	77.5	2.49	-
HbA0	90.25	80.58	-
HbA2	112	-	**1.27**
HbS-Window	120.25	0.83	-

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
	 **Figure 22: HbA2 / HbF assay chromatogram**	transparent red area In the HbA2/HbF assay, the HbF & HbA2 peaks are identified by transparent orange areas	
Fit to view		Allows to display the entire trace	button
Normal display		Returns to the initial zoom display	button
Information area			
Information zone		Under the trace area; the test information area shows details of alarms, flags, messages with little warning logos This zone is equipped of a scrolling bar to move up and down in the list to view all lines It also displays the total area of the sample	Information field
Buttons area			
Previous		Allows the user to navigate among the results traces Click on previous to get to the previous trace This button is only active when there are previous result(s) present	button
Next		Allows the user to navigate among the results traces Click on Next to get to the next trace	button

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		This button is only active when there are more result(s) following the current displayed trace	
FILTER		This button is used to recall results from database, even previous from the running day and can be searched by: • Patient, Controls or calibrators *(which includes actual calibrators, primers and blanks).* • Date range • Assay • Sample ID • Name	Button
LIS		This button is used for resending manually the results of the current sample to the LIS	Button
PRINT		Click on this button to print or reprint the result report	button

7.10. Quality Control Window Part I: Control Table



7.10.1. QC Window Highlight

Note that the Quality Control Window Icon is backed with grey to show that it is active and not selectable.

7.10.2. Assay Selection

To view the Controls which have been run for a particular assay e.g. HbA1c or HbA2, select the appropriate analyte using this combo box.

7.10.3. Control Results

The Control Results for the selected analyte are displayed in this table. Both Level 1 and Level 2 Controls are displayed here.

7.10.4. Add A Lot Button

To add a new lot of control for the selected analyte, press this button (see Section 7.12 for further details).

7.10.5. Levey-Jennings Buttons

The Levey-Jennings Button will display the Levey-Jennings screen (see Section 7.10.8 for more details).

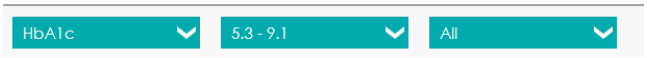
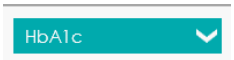
7.10.6. Flags / Alerts Icon

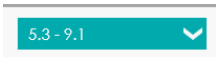
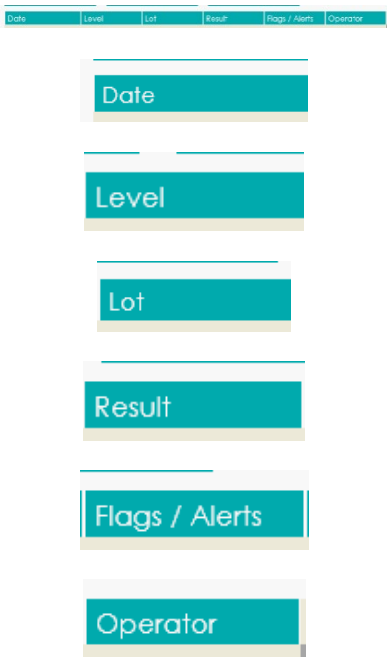
The Flags / Alerts Icon is placed next to any Control Sample with a Flag / Alert raised against it.

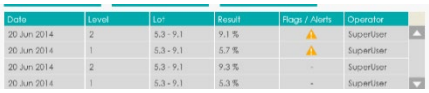
7.10.7. Print Button

The Print Button can be used to Print the entire QC Table on-screen.

7.10.8. Details of the Control table window

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
Selection area			
Assay selection		The assay selection is a combo box composed of the 3 different calibrated analytes of the system: HbA1c, HbF & HbA2 By default, it displays the first element of the list: HbA1c Clicking on the combo Box opens the list and the other 2 analytes can be selected Highlight the desired selection to display the related information	Combo box

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
Lot Selection		This field is dependent on the Assay selection (Previous combo box) Once the assay is selected the corresponding available lot numbers containing results are displayed. Click on the combo box to open the list Highlight one element of the list to select it	Combo box
Level(s) selection		This field is used to select the level(s) to be displayed. By default All is selected meaning that the 2 control levels of the selected lot are displayed Click on the combo box to open the list The list is composed of All, Level 1 and Level 2 Highlight the desired selection to restrict the content of the table to the selection	Combo box
Control table area			
Column titles		These column titles label the content of the tablet The date is the date Day Month Year of the Control results The Level displays 1 or 2 depending the QC level result The Lot displays the name of the lot Number The result displays the calibrated result for the main unit Flag / alert displays the flag in case of a problem; to find out the details of the flag/alert, see below Flag / alert explanation	Text / button combined area

FIELDS	DISPLAYED AS	DESCRIPTION / USE	FORMAT
		The Operator displays the ID of the user logged in at the time of the QC results calculation.	
Table Lines		The lines are created by each QC result calculation	table
Flag / Alert icons		This icon refers to either a QC data outside of the QC defined range or to a reaction alarm Click on the Flag /alert Icon cell to see the details of the flag for the specific QC data	icon
Buttons area			
LEVEY-JENNINGS		Allows the user to open the Levey-Jennings chart for the selected QC. Click on the Levey-Jennings button and the Levey-Jennings window will open	button
TRACE		Allows the user to view the chromatogram for the selected QC point Click on TRACE button and the trace will display under the list of result the trace of the highlighted result	button
ADD LOT		This button is used for the creation of a new QC lot. Clicking on this button will open the ADD LOT dialogue	Button
PRINT		Click on this button to print or reprint the result report for the selected QC point	button

7.11. Quality Control Window Part II: Levey-Jennings

The QC Window's Levey-Jennings' screen allows the view and review of Control sample results plots over time. This allows for the tracking of instrument control to ensure results for patient samples will be within stated accuracy limits.

7.11.1. QC Window Highlight

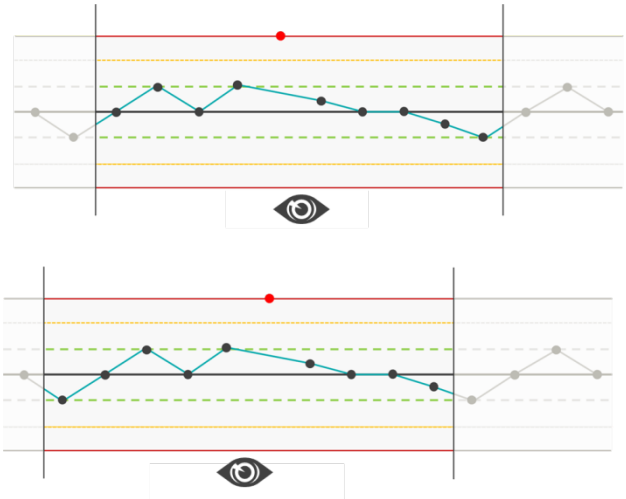
Note that the Levey-Jennings' screen is within the Quality Control Window, so the Quality Control Window Icon is still backed with grey to show that it is active and not selectable.

7.11.2. Assay / Lot / Level Selection

These three combo boxes require the selection of a specific Lot and Level for a Control assay type in order to display data for that group. Pressing each combo box will only allow the selection of Assays, Lots and Levels, which have been added to the system.

7.11.3. Pan Backward Button

Data is displayed in batches of ten consecutive Control results. The Pan Backwards Button will scroll back through the Controls results one by one every time it is pressed, as shown below:



7.11.4. QC Main Button

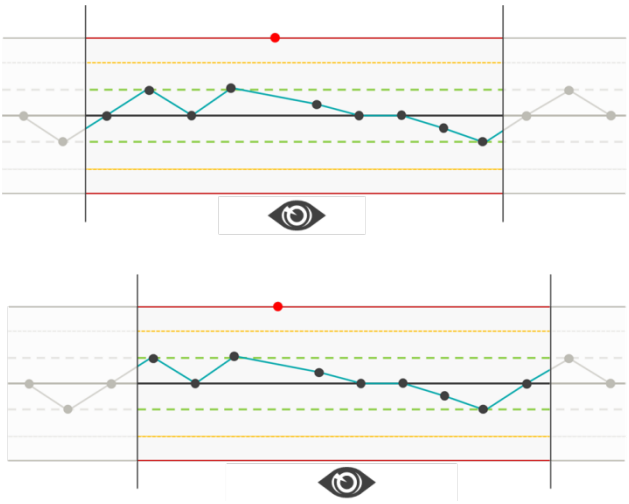
The QC Main Button will take you out of the Levey-Jennings screen and back into the main QC Window.

7.11.5. Levey-Jennings' Button

Note that since the Levey-Jennings screen is currently displayed the Levey-Jennings Button cannot be pressed again and is therefore inactive / grey.

7.11.6. Pan Forward Button

Just as with the Pan Backwards Button, the Pan Forwards Button will scroll forward through the Controls results one by one every time it is pressed, as shown below:



7.11.7. Control Result

Each single control result is selectable. Pressing a control result will bring up a Result Dialogue which will provides the details of that result, and allows it to be rejected / re-accepted if necessary:

Control results which are highlighted red are out of the currently displayable range of the Level-Jennings Chart.

7.11.8. Print Button

The Print Button allows the Levey-Jennings chart currently on-screen to be printed. This printed chart will only contain the results currently on-screen. It remains disabled if no graph is displayed on-screen.

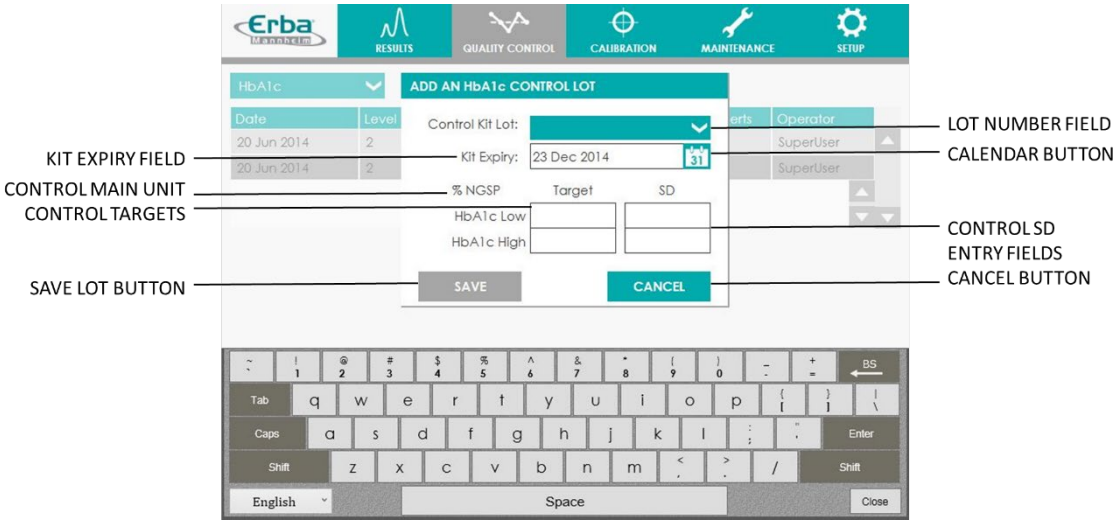
7.11.9. Statistics Button

The statistics button provides information on the performance of the selected lot of control material by way of an overall mean, standard deviation and CV. Rejected controls are removed from these statistics.

The statistics box will revert to the closed position if the Levey-Jennings screen is exited and then re-entered.

7.12. Quality Control Window Part III: Add A Lot Dialogue

The Add A Lot Dialogue allows QC Lots to be added for a specific assay. To determine which assay to add a lot for, select the desired assay using the combo box in the top left of the QC Main Window before pressing the Add A Lot Button.



7.12.1. Kit Expiry Field

The Kit Expiry Field allows for the entry of a Kit Lot expiry date. Pressing this field brings up the virtual keyboard. Alternatively the Calendar button can be pushed (see section 7.12.6 for details).

7.12.2. Control Unit Information

This information in front of the Target and SD titles give the user the information of the main unit, in order to direct as to which values to enter.

7.12.3. Control Target Field

The Control Target field provides space for the Control Mean to be entered.

7.12.4. Save Lot Button

The Save Button is used to save the lot information provided for this new lot. It is only active once all of the required fields have been filled out.

7.12.5. Lot Number Field

The Lot Number Field allows for the entry of the Lot number of the Control material being added to the system. It also allows the selection of an existing lot number from the editable combo box.

7.12.6. Calendar Button

Control Lot Expiry can be added by pressing the field and using the on-screen keyboard or by pressing the Calendar button and selecting the data using the pop-up Calendar Dialogue.

7.12.7. Control SD Entry Fields

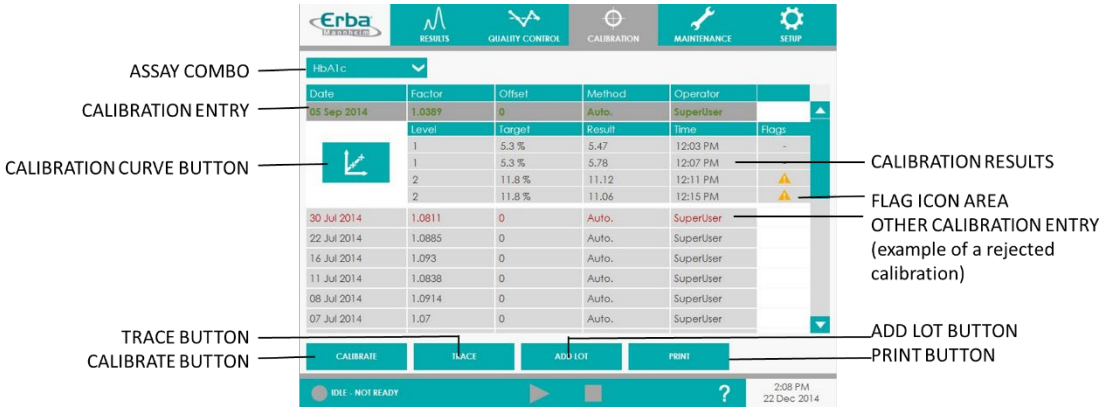
The Control SD Field allows for the entry of the Standard Deviation about the mean. These are used to determine acceptability limits for the control lot (Mean +/- 2SD)

7.12.8. Cancel Button

Cancels the adding of a new lot and closes the Add A Lot dialogue.

7.13. Calibration Window I: Calibration Table

The Calibration Window allows the system to be calibrated, and allows the review of calibration data and calibration samples.

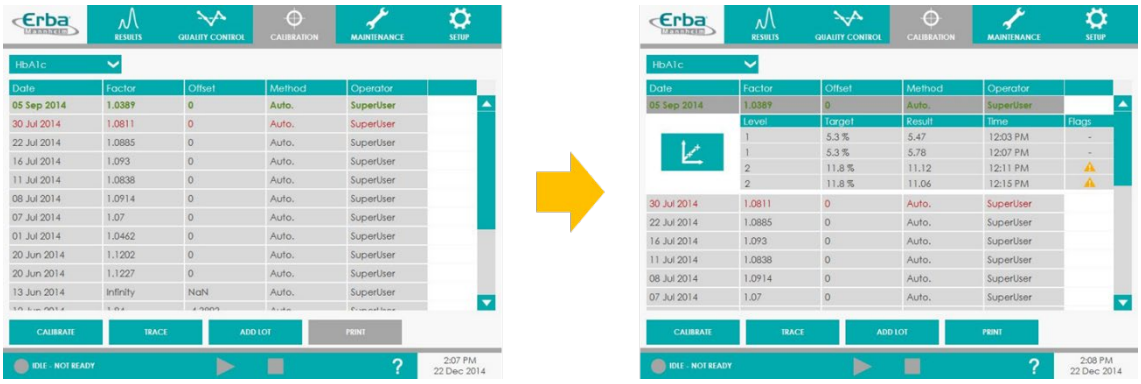


7.13.1. Assay Combo Box

The Assay Combo Box provides a method of selecting which assay's calibration data is being viewed on screen at any time. This selection should be made before pressing either Add A Lot Button or the Calibrate Button as the setting of this box determines which assay is being calibrated or for which assay a lot is being added.

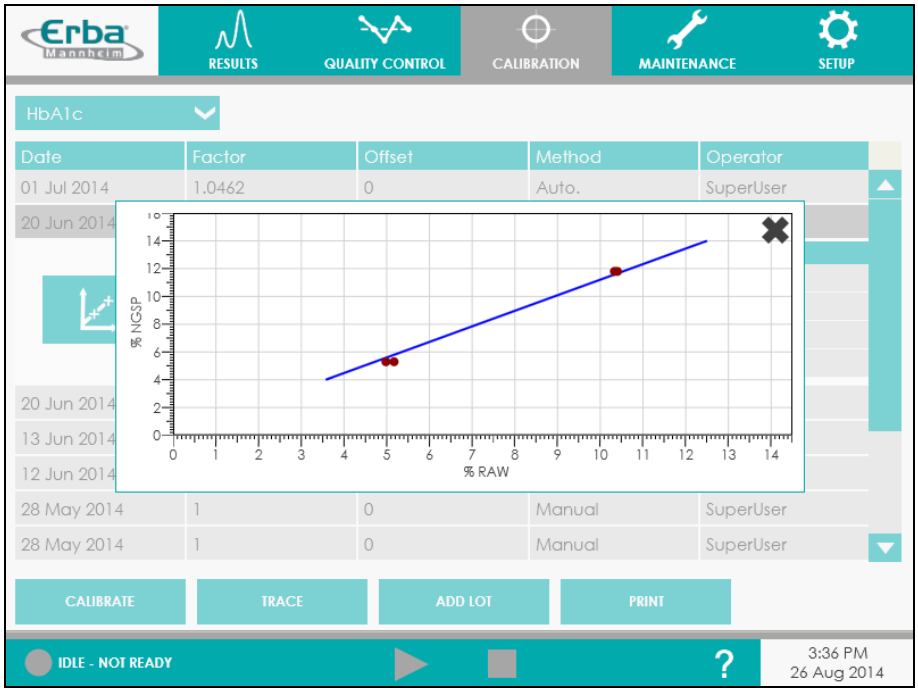
7.13.2. Calibration Entries

Calibration entries can be selected by pressing the row. This opens up the dropdown displayed above which provides access to the Calibration Curve Button and the results for the individual calibrator levels run for this calibration:



7.13.3. Calibration Curve Button

The Calibration Curve Button provides access to the Calibration Curve Dialogue box, displaying the curve the calibration selected:



The calibration curve window is closed by the X on the upper right corner

7.13.4. Calibrate Button

The Calibrate Button brings up the Calibration Dialogue, via which calibration data is added prior to a calibration.

7.13.5. Trace Button

The Trace Button displays the chromatography for the currently selected calibrator sample.

7.13.6. Add A Lot Button

The Add A Lot Button opens the Add A Lot dialogue for adding calibrator lots to the system.

7.13.7. Print Button

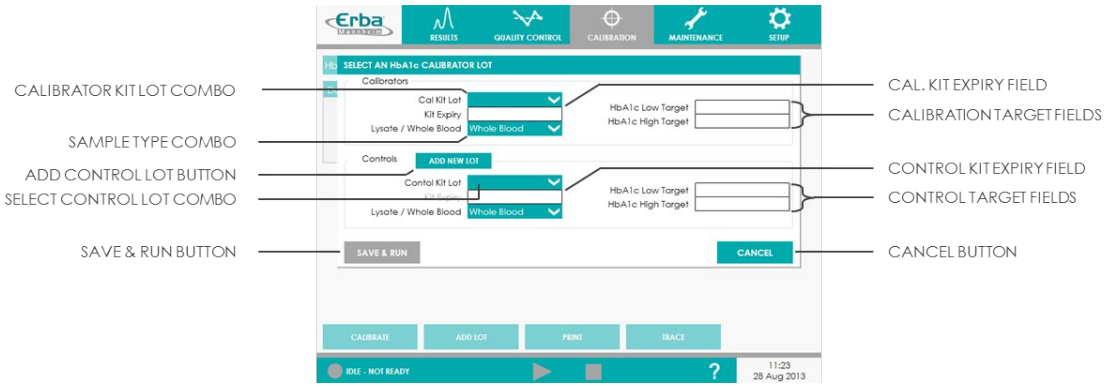
The Print Button allows the printing of the currently displayed Calibration Table.

7.13.8. Calibration Results

The Calibration Results are the results for the individual calibrator levels runs in a calibration run.

7.14. Calibration Window Part II: Calibrate Dialogue

The Calibrate Dialogue allows for the entry of data required for a calibration run to be performed:



7.14.1. Calibrator Kit Lot Combo Box

The Calibrator Kit Lot Combo Box allows the selection of a Calibrator Kit Lot from a dropdown list.

7.14.2. Sample Type Combo Box

The Sample Type Combo Box allows the Calibration to be run as whole blood samples or lysate samples.

7.14.3. Add Control Lot Button

Provides the Add Control Lot dialogue directly from this screen in case the user forgot to add their control lot before navigating to this screen.

7.14.4. Select Control Lot Combo Box

The Select Control Lot Combo Box allows the selection of a valid Control Kit Lot from a dropdown list. Only Control Lots which are within expiry will be displayed.

7.14.5. Save & Run Button

The Save & Run Button with display the Status Window with all of the relevant tube positions already filled out for the user.

7.14.6. Calibrator Kit Expiry Field

The contents of the Calibrator Lot Target Fields are linked to, and determined by, the Lot selected in the Select Calibrator Lot Combo Box.

7.14.7. Calibration Target Fields

The Calibration Target Fields' content are linked to, and determined by, the Lot selected in the Calibrator Lot Combo Box.

7.14.8. Control Kit Expiry Field

The Control Kit Expiry Field's content is linked to and determined by the Control Lot selected in the Select Control Lot Combo Box.

7.14.9. Control Lot Target Fields

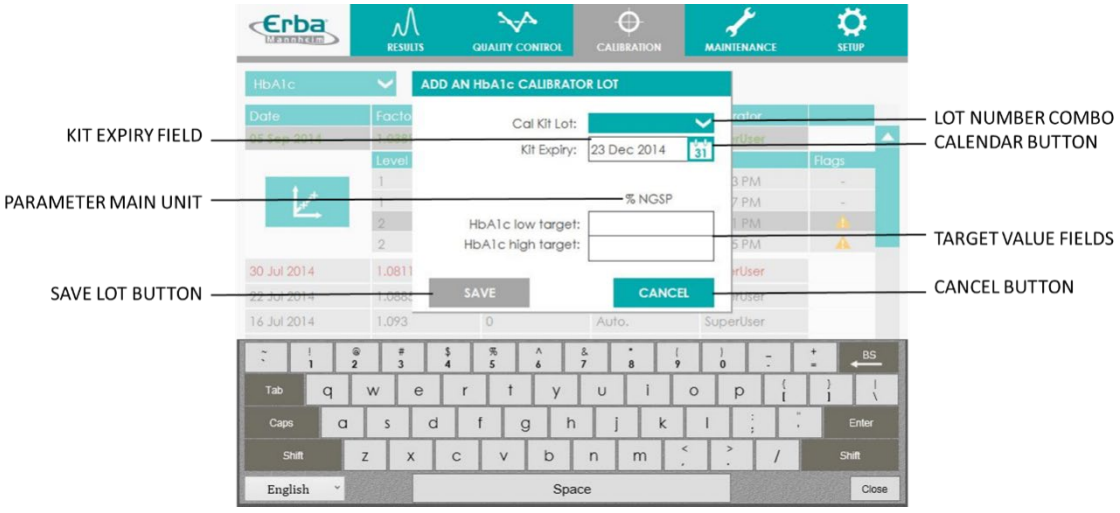
The contents of the Control Lot Target Fields are linked to, and determined by, the Lot selected in the Select Control Lot Combo Box.

7.14.10. Cancel Button

The Cancel Button closes the Calibrate Dialogue and abandons the calibration.

7.15. Calibration Part III: Add Lot Dialogue

The Add A Lot Dialogue allows Calibrator Lots to be added for a specific assay. To determine which assay to add a lot for, select the desired assay using the combo box in the top left of the Calibration Window before pressing the Add A Lot Button.



7.15.1. Lot Number Combo

The Lot Number combo allows for the entry of the Lot number of the Calibrator material being added to the system as well as recalling existing lot numbers.

7.15.2. Kit Expiry Field

The Kit Expiry Field allows for the entry of a Kit Lot expiry date. Pressing this field brings up the virtual keyboard.

7.15.3. Parameter Main Unit

The parameter main unit is an information provided to guide the user when choosing the correct value to be entered.

7.15.4. Calendar Button

Calibrator Lot Expiry can be added by pressing the field and using the on-screen keyboard or by pressing the Calendar button and selecting the data using the pop-up Calendar Dialogue.

7.15.5. Calibrator Target Fields

The Calibrator Target Fields provide allow for the entry of Calibrator target values. These are found on the Calibrator Kit Insert sheet.

7.15.6. Save Lot Button

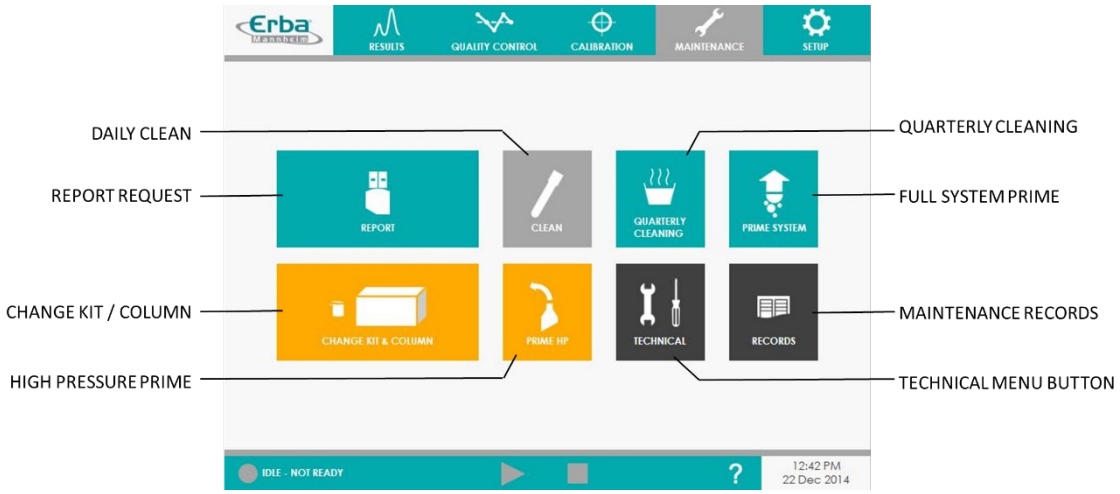
The Save Lot Button is used to save the lot information provided for this new lot. It is only active once all of the required fields have been filled out.

7.15.7. Cancel Button

Cancels the adding of a new lot and closes the Add A Lot dialogue.

7.16. Maintenance Window

The Maintenance Window provides access to both 'soft' maintenance options for end users, as well as access to full maintenance options designed for service engineers.



7.16.1. Color Coding of Buttons

The Hb-Vario has a complete Maintenance Window which provides simple and convenient access to maintenance features for end users. Initial orientation on this window can be found in section7.16.

This section covers the use of each of the buttons in the Maintenance Window in context to provide greater clarity as to their specific usage. The Maintenance Window is broken into colored tiles for convenience. Teal / Blue tiles are 'soft' maintenance items which are expected to be used by end users. Yellow / Orange tiles are related to the replacement of consumable items. Dark Gray tiles are related to technical, 'hard', maintenance items and are expected to be required by trained technical service personnel.

7.16.2. Report Request Button

The Report request Button provides end users the possibility to export data. It can export data of 1 test or a range of tests: The export process can include the pdf reports of the tests, but can also consist of raw data, logs and the database.

The whole content is placed at the root of the USB key and can be used to report technical issues to the technical service or backup results.

The file name of the pdf export is for example **00001-010-20160401-Report-10-100.zip**
There are as many PDF files in it than the number of tests within the selected range.

- Where 00001-010 represents the Serial Number of the instrument, *(it can also be of format HBV-E0001 or HBV-D0001)*
- 20160401 is the date in YYYYMMDD
- Report contains the pdf exports (same as printouts from instrument)
- And where 10-100 represents the export of test number 10 to 100.

The file name of the data export is for example **00001-010-20160401-Datas_Export-10-100.zip**
Where the details are as above except for:

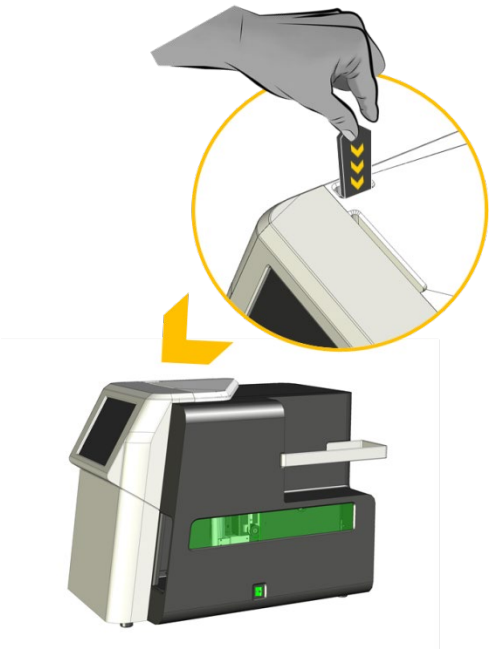
- Datas_Export contains the data export (for support or backup) this includes the database, the result data and the logs

To request report:

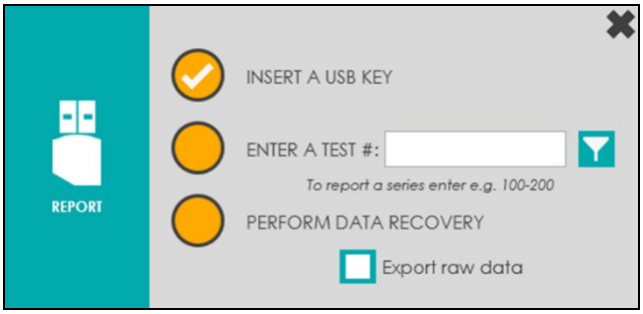
1. Start by pressing the report button 

in the Maintenance Window. This will display the Report dialogue.

2. Once in the Report dialogue, insert a suitable USB drive in the Hb-Vario's USB slot in the front of the instrument.

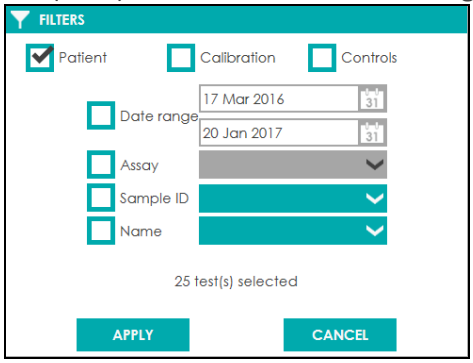


3. Confirm that the USB has been installed, by clicking on the orange circle INSERT A USB KEY.



4. Select the test range:
   a. Enter the Test# or range of Test#'s you wish to recover:
      - in the text box, e.g. 100 – 200 to recover Test#'s 100 to 200.

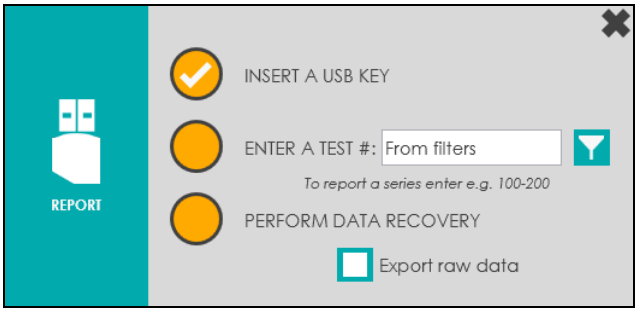
   b. Or Click on the (filter) button to access the filtering options:



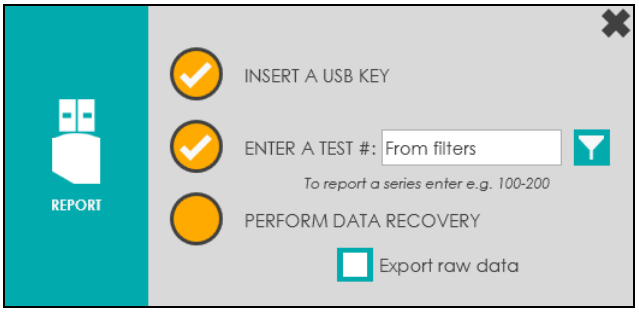
- From there check the box(es) in front of the filter(s) you wish to select:
  - the type(s) of results you wish to recover:
    - patient, calibration, controls
  - And/or a date range can be selected

- Or sample ID or name can be filtered
- Then click on APPLY button to validate them

The Filter dialogue box will close and the following window appears



5. Confirm the Test range to be recovered by clicking on the orange circle in front of the ENTER A TEST #. *A check mark will appear in the circle*



6. The selection of pdf reports or data is done at this point:
   a. To recover pdf reports:
      - Confirm that you are ready to recover by clicking on the orange circle in front of PERFORM DATA RECOVERY

   b. To recover data (database, raw results data and logs):
      - Check the Export raw data box
      - Confirm that you are ready to recover by clicking on the orange circle in front of PERFORM DATA RECOVERY
7. Wait for the recovery to complete. The Report dialogue will close and return to the Maintenance Window upon successful completion.

Note: In case both reports and data are needed to be recovered, perform 2 exports, 1 for reports, and 1 for data afterwards.

If exported once, the pdf reports (of the selected range) are saved to the disk, subsequent exports of the same reports will export the pdf created initially.

Warning: Regular external Data backup or export is highly recommended to secure data integrity.

For this function the REPORT button can be used, and data selection (test range) can be 0-9999.

7.16.3. Clean Button

The Hb-Vario's clean cycle can be either initiated from the status window by placing and declaring the cleaners on-board (see 7.5.3Rinse&Cleaner entry for more details), or from the Maintenance Window at the end of every day.

From the maintenance window, 2 types of cleaning cycles can be initiated:

- A Clean and Power off
- A comprehensive Clean

Placing the cleaner on-board the rack during a run will perform the comprehensive cleaning procedure.

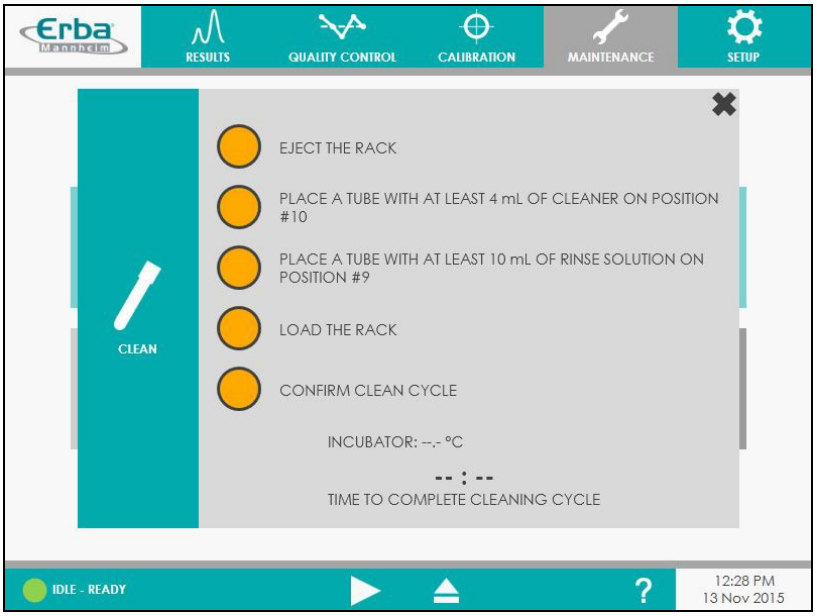
The clean and Power Off, will aspirate the cleaner and leave it in contact overnight while the instrument is turned off. The rinsing takes place in the morning upon restarting the instrument.

The Comprehensive clean performs a heated cleaning that last around 20 minutes and leaves the instrument ready to go for the next run.

Press the Clean button in the Maintenance Window. This will open a submenu with selection of the desired option

Selecting the either clean option will cause the Hb-Vario rack holder to be ejected ready to hold a sample rack with a Cleaner and Rinse.

Follow on screen instructions, for more details consult 8.1.2.1.1 Cleaning procedure from Maintenance menu



Note: The rack must be empty to perform this action.

Prepare a Cleaner tube by adding a minimum of 4 ml of Hb-Vario Cleaner to a Regular Primary (13 x 75 mm) tube. Place this is in the last sample position (10) on the rack. Prepare a tube “10 ml” tube provided in the Hb-Vario kit or in the cleaner kit with at least 10 ml of the Rinse or cleaner 2 in position 9 and place the rack on the Hb-Vario rack holder.

Warning: Be sure to remove the sample tube caps before inserting the Hb-Vario Cleaner sample into the system. Sampling from capped tubes / vials will result in damage to the sample probe.

Confirm that you have loaded the Cleaner sample. The instrument will then accept the rack holder and rack. Confirm that you are ready to run the Cleaner Sample and wait until the timer expires.

7.16.4. Quarterly Cleaning Button

The quarterly cleaning Button allows the user to perform the Quarterly Maintenance.

This performs a hot water cleaning of both low and high pressure systems. This procedure lasts about 14 minutes. It should be performed on a kit that has reached 100 tests, as running the hot water through the column will damage it, and the kit will be automatically deactivated and wasted if less than 100 tests were run. See 8.4 Quarterly maintenance for more details on how to operate the cleaning.

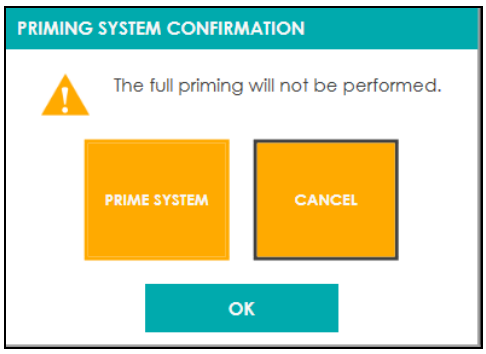
7.16.5. Prime system

Priming the Hb-Vario draws reagent from the reagent bottles into each of the pumps High Pressure Pump A, High Pressure Pump B and Hemolyser Peristaltic Pump.

Warning: This procedure is intended to prime the system **during initial installation or resuming activity after storage under specific storage fluids**. It is used to drawn the storage solutions out of the system and replace them by working reagents (Hemolyzer, Reagent A and B). By doing so the reagent kit and column will be damaged. Once completed the cycle, the kit used for this procedure will be disabled. A new kit will have to be installed to start normal operations.

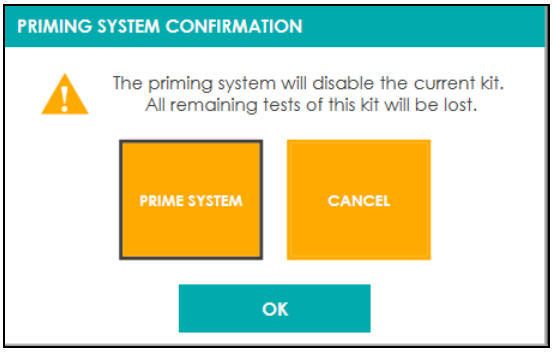
1. Upon clicking on the Prime System button 

The following confirmation popup will open. *Since this procedure damages the kit, the pre-selection will be on* **CANCEL** *for security.*



- Click on OK to confirm Cancellation. *The Step will display with a Failed mark* 

2. If the Priming System is required, select the Prime System option

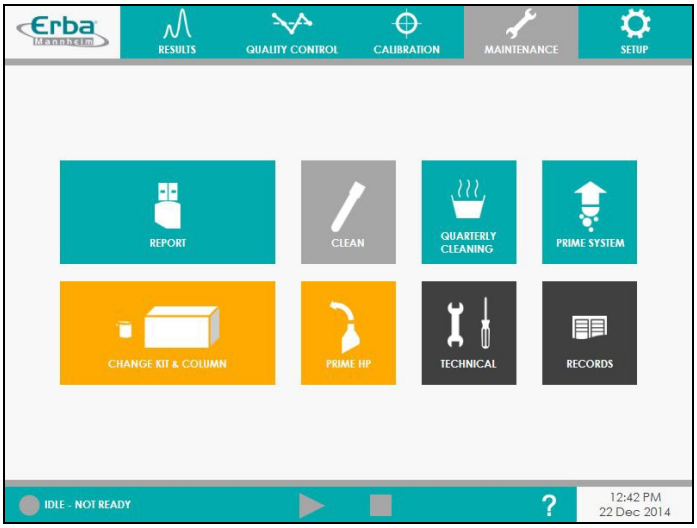


3. Then click on OK to confirm

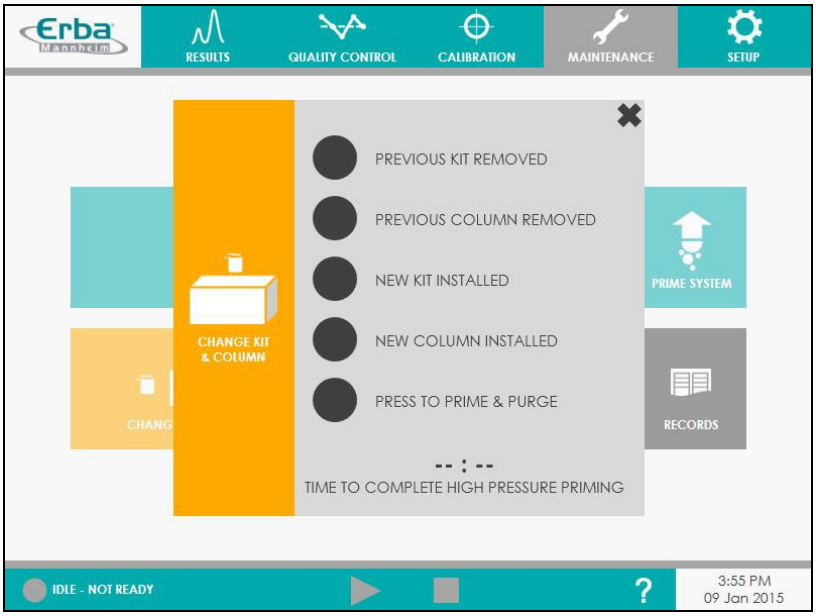
4. The procedure dialogue box appears, follow on screen instructions as well as the time left to completion.

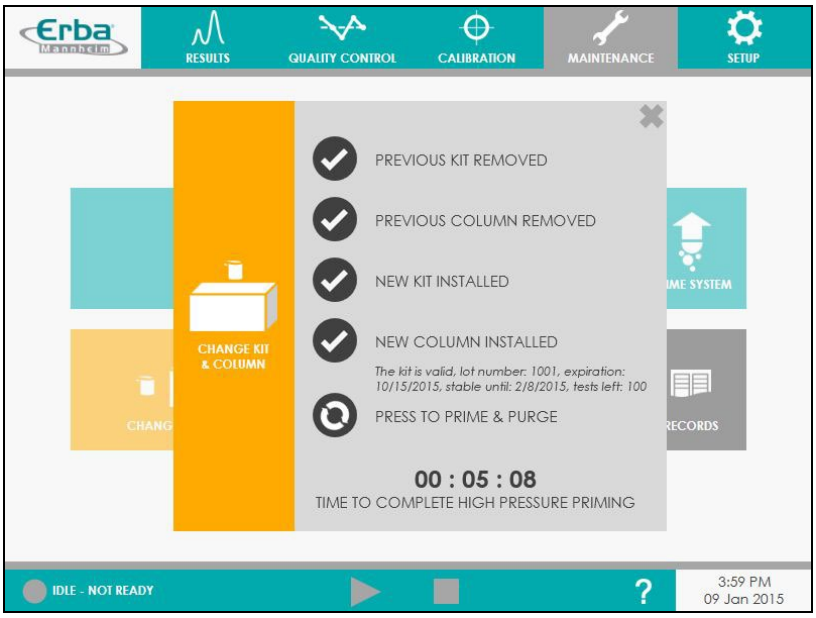
7.16.6. Change Kit & Column Button

Using the Top Menu, select the Maintenance Window as shown below.



- Then press the ‘Change Kit & Column’ option at the bottom left of this screen.
- Follow the on-screen instructions, confirming at each step that there is no previous kit or column attached to the system and that the new kit and column have been installed.

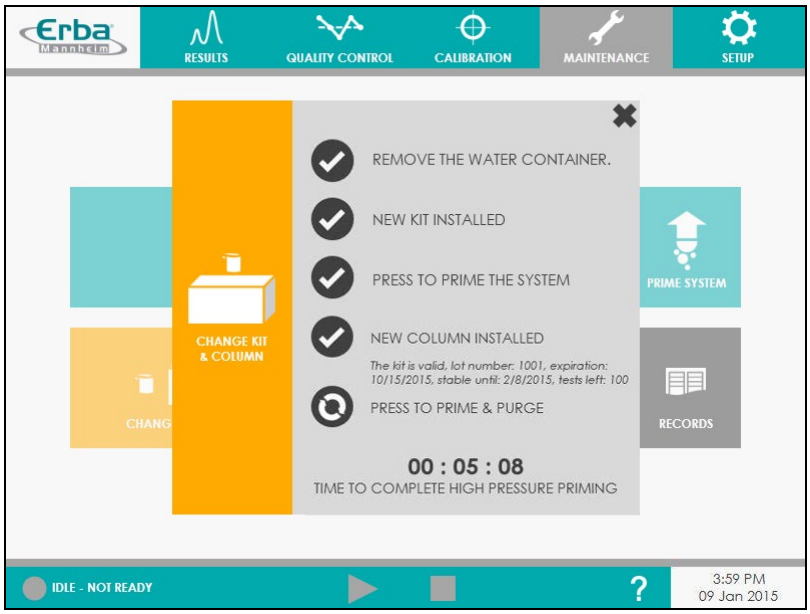




Once the new column installed confirmation box has been checked the system will authenticate the kit and column before requesting priming and purging the system. (Also refer to 4.5 kit registration for all details)

If Change Kit & Column is selected immediately after completing Prime System then the following box appears on-screen;

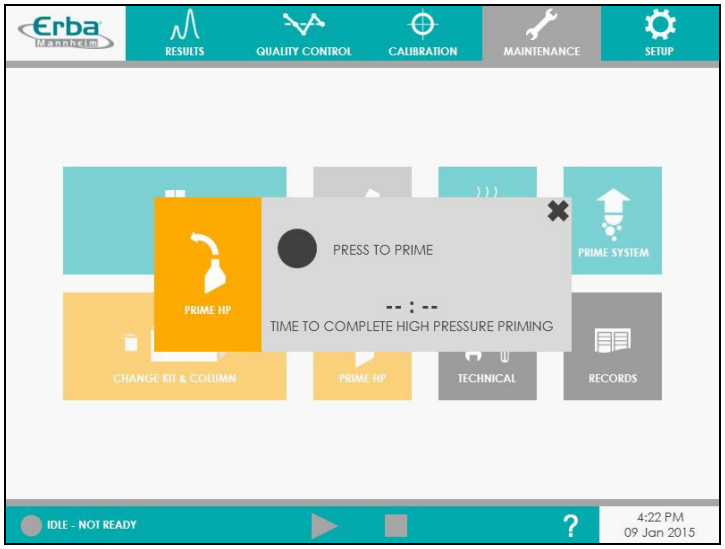
Follow the on-screen instructions, confirming at each step like removing the water container, installing the new kit and column and priming & purge.



Once the new kit and column are installed and the confirmation box has been checked the system will authenticate the kit and column at the respective steps before requesting priming and purging to the system (Also refer to 4.5 kit registration for all details).

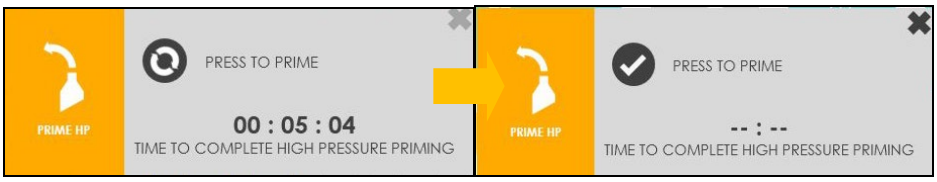
7.16.7. Prime HP (High Pressure Priming)

This cycle allows priming of both High Pressure pumps (A&B). Click on the Prime HP button to perform this prime of A and B, follow the on screen instructions, shown below. The full process takes about 6 minutes.



1. Click on the Press to Prime instruction circle,

The system will display a rotating double arrow, until the process is completed, confirmed by the check mark as shown below.



7.16.8. Technical Menu Button

The Technical Menu is a partially restricted area for some sections (make Adjustment). It is mainly designed for the service engineers.

Technical is accessed from the main maintenance window by clicking on the Technical button

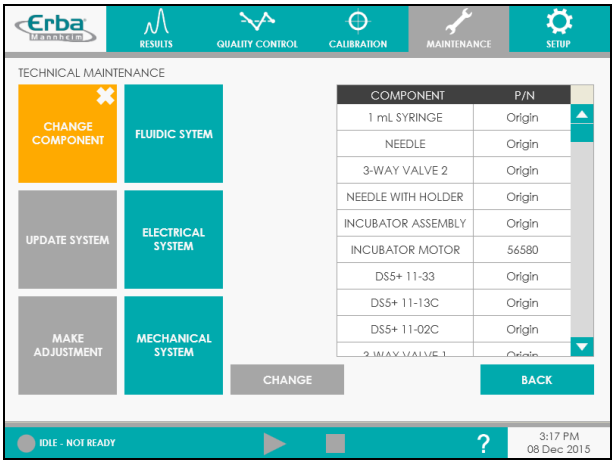
It is composed of 3 sections:

1. Change component
2. Update system
3. Make Adjustment

7.16.9. Change component functions:

Once in the Change component section, a list of component displays in a table on the right of the window. The list is dynamic and displays all the items if no further selection is made. The change component is itself classified in 3 categories:

1. Fludic system
2. Electrical system
3. Mechanical system



Once a category is selected, the component table restricts to that category.

To close the window click on the BACK button.

Note: Access to Change component is limited to view for users with USER rights, and the button CHANGE can be accessed with ADMIN rights. See different access rights for details.

1. Fluidic system:

This section is divided in 3 types:

- Sample Aspiration
- Sample preparation / waste
- High pressure

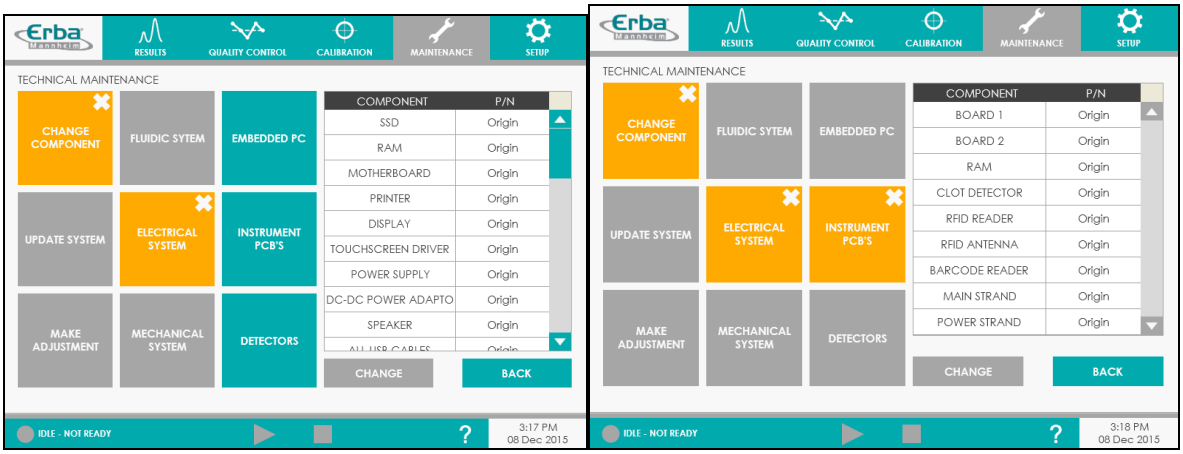
The resulting component gets further and further restricted as the categories and types are selected.

2. Electrical system:

This section is divided in 3 types:

- Embedded PC
- Instrument's PCB
- Detectors

The resulting component gets further and further restricted as the categories and types are selected.

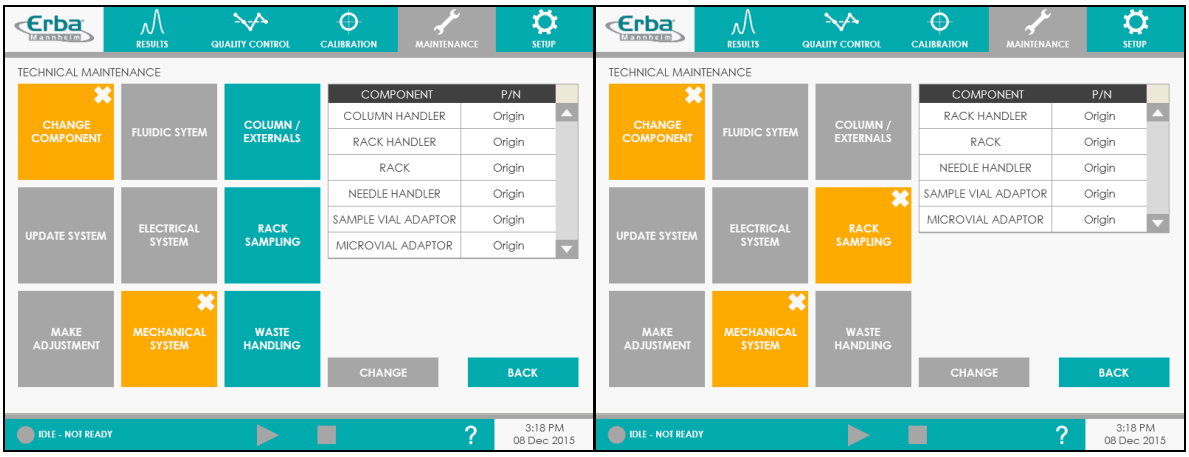


3. Mechanical system:

This section is divided in 3 types:

- Column / Externals
- Rack Sampling
- Waste handling

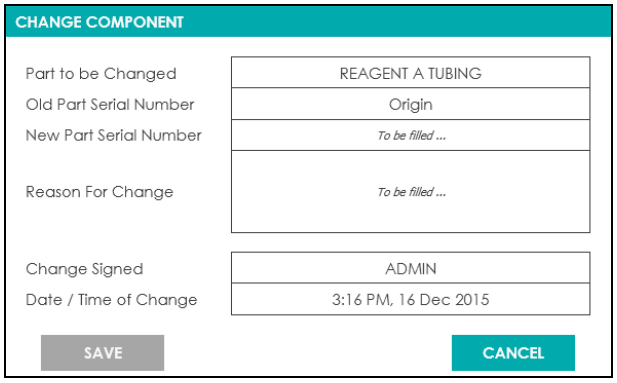
The resulting component gets further and further restricted as the categories and types are selected.



7.16.10. Change component input pop-up:

Upon selection of an item from the component table list, the CHANGE button becomes active.

Click on the CHANGE button to open the CHANGE COMPONENT window.



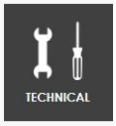
This window allows detailed information to be saved into the system for traceability. It would allow recalls of specific part/serial number/batch

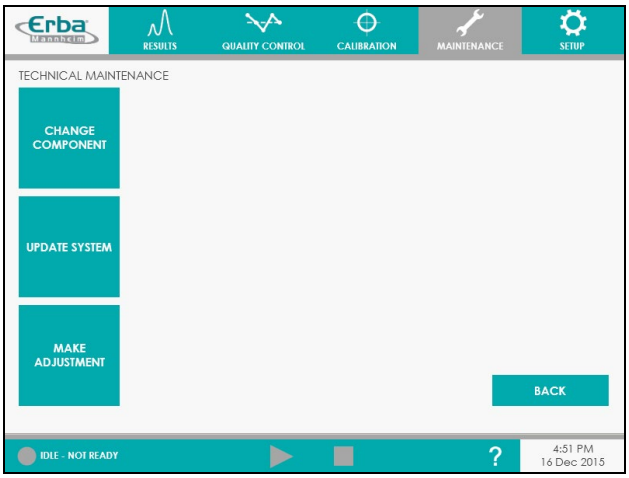
Fill all the requested information to activate the SAVE button.

7.16.11. Update System function

This button can be used to upgrade the system by USB. ***Only an ADMIN user can access the software update function.***

1. Click on the Maintenance button from the Top Menu 

2. Click on the Technical button 

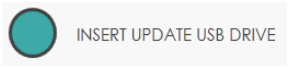


3. Click on the UPDATE SYSTEM button. There are two options are available: UPDATE SOFTWARE and UPDATE CONFIG FILES

4. Click on the UPDATE SOFTWARE button

Upon selection of the update software button the dialog area appears.

5. Follow the steps by inserting the USB key on which the software update files have been copied in any available USB port of the instrument, then clicking on the step
   a. The instrument will check for presence of a valid key,
   b. if it fails to find a key connected it will display a for that step,
   c. otherwise
6. Upon status of first step, selected “Press to Update” step to confirm your intention.
7. A message will pop-up:*“Are you sure you want to update the software? The system will reboot to perform the update.”* Yes /No
   a. If No is selected, the procedure is aborted and step displays failed status .
   b. If Yes is selected: Step changes to processing status and Instrument checks the presence of valid update files and performs update, ending in rebooting the system.
   c. If update file is not found, it will lead to a failed status
8. Click on the UPDATE CONFIG FILES button.

9. Select 

to update configure files by inserting USB drive
10. Follow above steps 5 to 7 to update configure files.

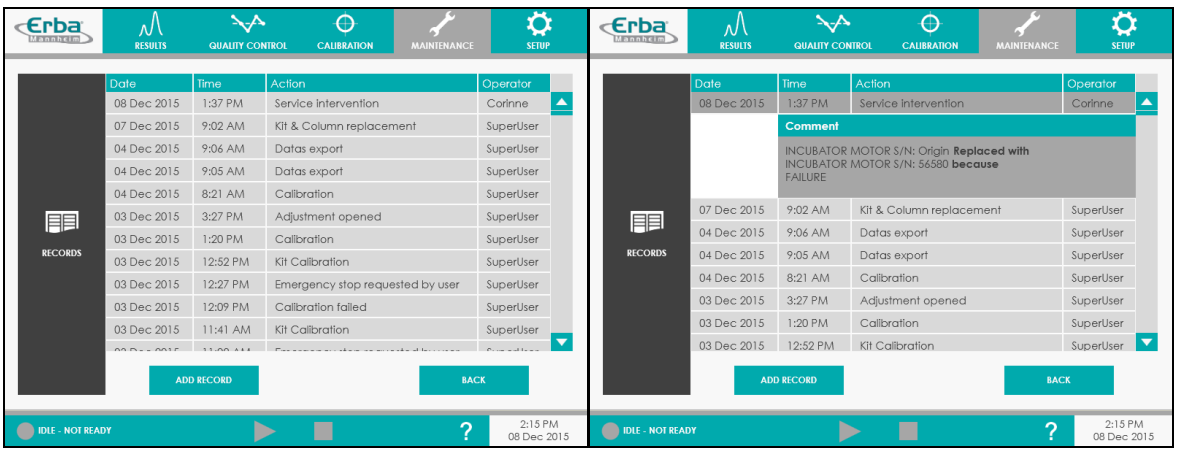
7.16.12. Make Adjustment function

This section allows full technical service and intervention on the instrument and is covered in detail in the Hb-Vario's Service Manual. This function is restricted and can only be accessed with specific passwords. End users do not have access to this menu.

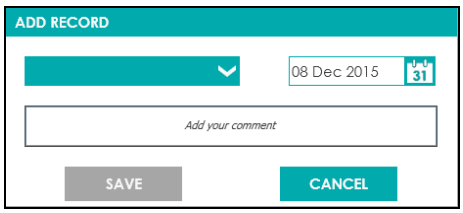
7.16.13. Records Button

The Hb-Vario's Maintenance Records system will record all of the following service interventions on the system to ensure full traceability and aid technical service troubleshooting on the instrument:

- Kit & Column Change
- Calibration
- System Error e.g. part failure detected
- Service Intervention e.g. Valve Cleaning, Tubing Change or Leak Fix
    - Service Interventions require a comment to be made as to the problem and the solution.



To manually add a record, click on the ADD RECORD button at the bottom of the Records window.



It opens the ADD RECORD window. Select between *Preventative maintenance* and *Other* from the drop down menu, then fill the requested info to activate the SAVE button. Click the SAVE button.

Once the record is saved, it can be viewed in the record list as the latest record.

7.17. Setup

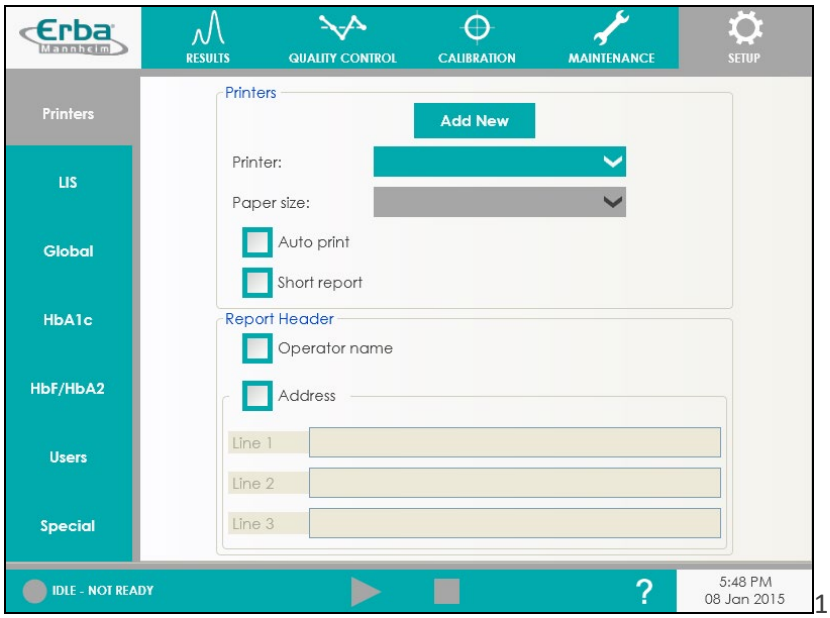
Under Setup 

The system can be configured for the laboratory use. The settings include:

- Printers
- LIS
- Global
- Assays (HbA1c and HbF/HbA2)
- Users
- Special

The details are explained here after.

7.17.1. Printers



1

From this window

- New printers can be added
- Paper size
- The laboratory can decide whether the results will be automatically printed as they get calculated, and either as a full or a short report
- The header can also be configured from here. If the Operator name and Address is checked in the corresponding check-boxes then the same will be printed in the final printout. A Maximum of 50 characters is accepted per address line.

Note: only ADMIN rights users can change the printers settings, click here for more details

7.17.2. LIS

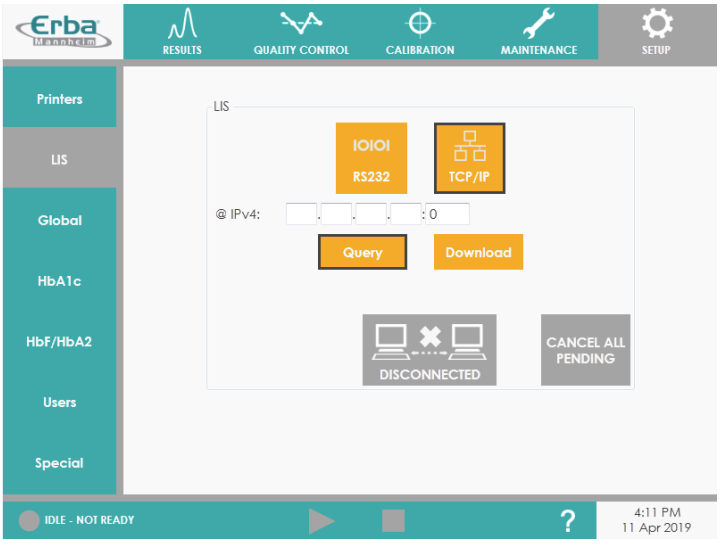


Figure 23: LIS configuration for Ethernet (TCP/IP)

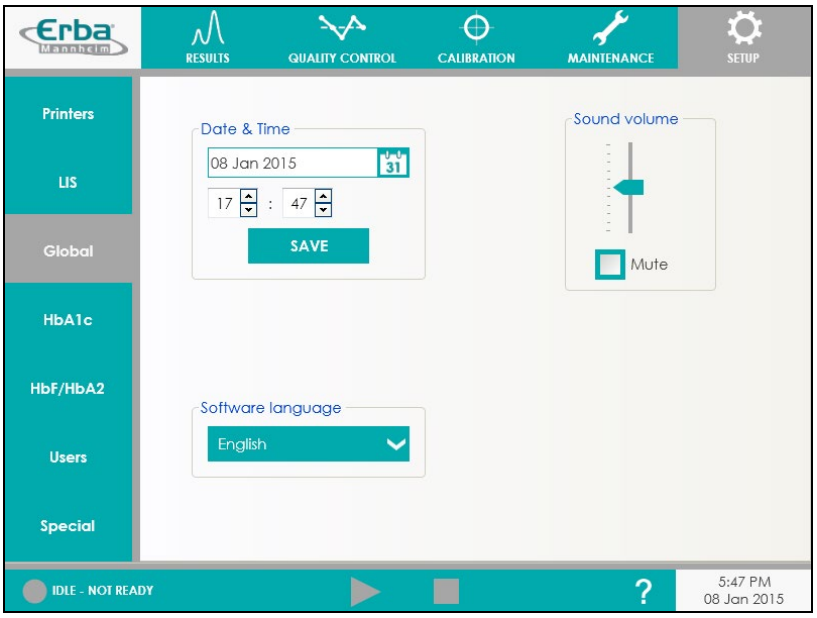
Figure 24: LIS Connection for Serial (RS232) mode

From this menu, the LIS can be configured***(only ADMIN rights users can change the LIS settings)***:

- It can work under serial connection or TCP/IP (Ethernet); clicking on either option will give the details of what needs to be configured for the specific communication
- Communication can also be achieved by download or query
- A button also allows to connect or disconnect with the LIS
- Cancel all Pending allows user to clear LIS queue.

Note: As soon as 1 of the 2 options is selected (black outlined), the LIS is considered activated, and the system will not program default analysis and replicate but await LIS requested tests. To deactivate LIS connection, click on the selected button option to remove the black outline.

7.17.3. Global



From the Global menu the user can configure:

- The Date and Time, click the SAVE button to save the changes
- The Sound level or mute the system
- Change/ select the software language

7.17.4. Assay menus

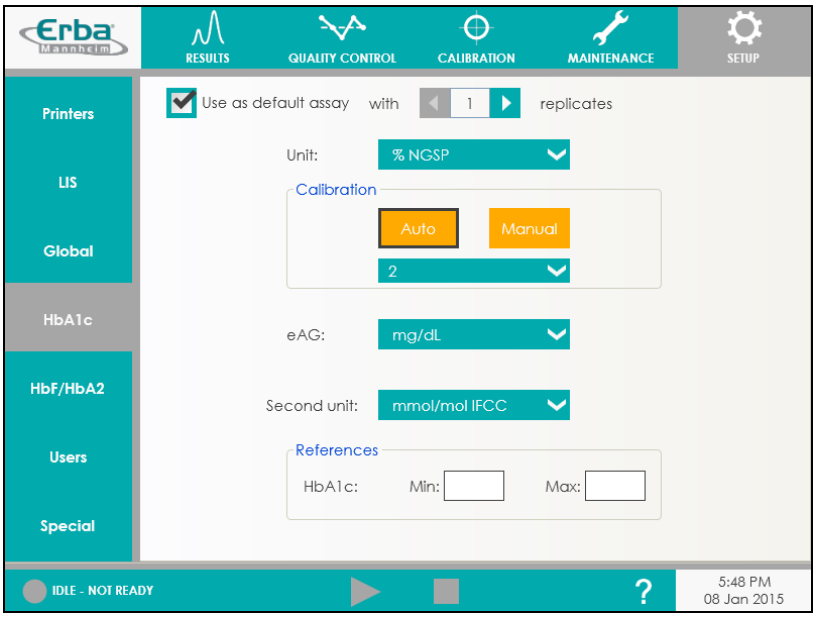
The assay menus HbA1c and the HbF/HbA2 give the configuration of the 2 types of assay that the Hb-Vario can run. ***Only ADMIN rights users can change the assay settings, click here for details.***

It is also where the default parameters are selected and saved.

The user can choose to use **one assay as default**, only one of the 2 can be, therefore once one is selected, the other one is automatically deselected.

The **number of replicates** is also selected from this window. This number will be applied to both assays, but can be modified during the test request. It will only apply for patients.

7.17.5. HbA1c

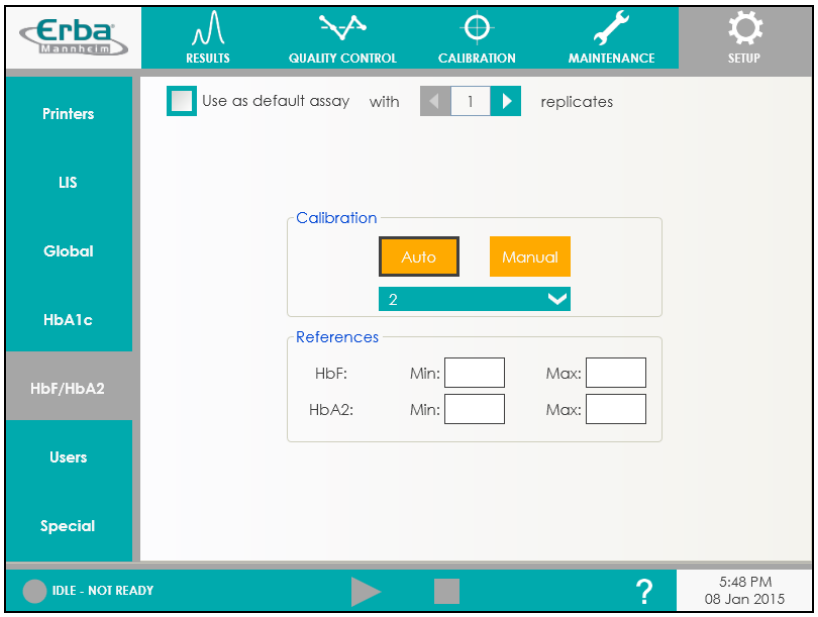


From the HbA1c, the user can select and configure

- The assay by default that will be used by the system
- The number of replicates (that will be applied commonly to both assays)
- The main unit between % NGSP, % IFCC, mmol/mol IFCC, % Mono-S
- The calibration type (auto or manual)
- The number of replicates of the calibrators (2 or 3)
- The optional report of eAG calculation and its unit–Estimated Average Glucose
- The optional second unit (from the left over list of available units)
- The laboratory specific reference range for the HbA1c in the main selected unit

Note: **Min.** value should not be greater than or equal to **Max.** value, else the input is not accepted. Also, maximum eight digits can be entered.

7.17.6. HbF/HbA2

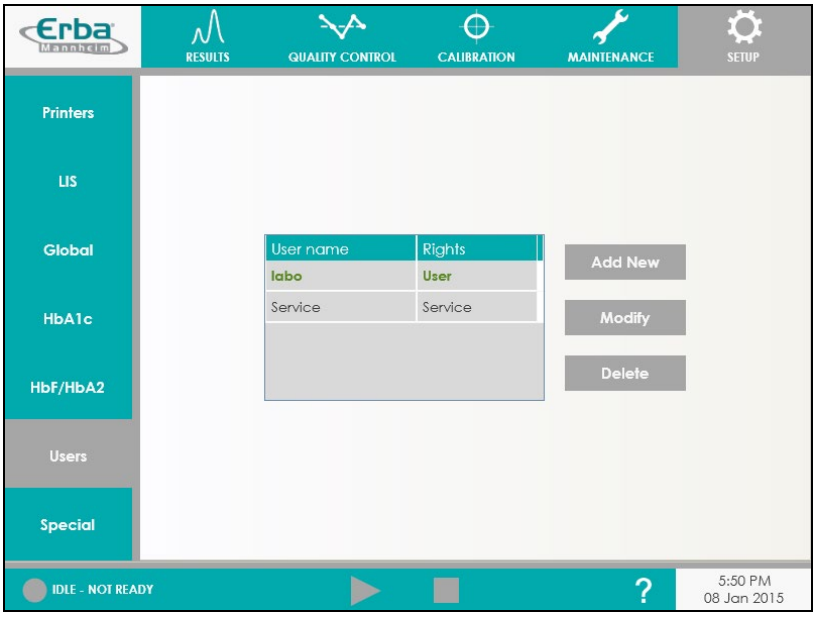


From the HbF/HbA2 menu, the user can select and configure

- The assay by default that will be used by the system
- The number of replicates (that will be applied commonly to both assays)
- The calibration type (auto or manual)
- The number of replicates of the calibrators (2 or 3)
- The laboratory specific reference ranges for the HbF and the HbA2

Note: **Min.** value should not be greater than or equal to **Max.** value, else the input is not accepted. Also, maximum eight digits can be entered.

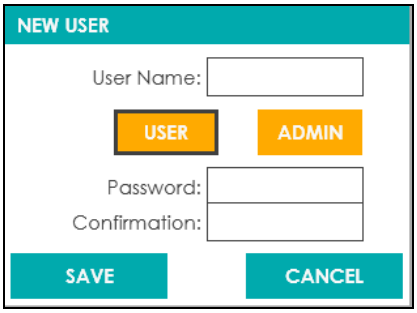
7.17.7. Users



- The user that is currently connected to the system appears in Bold Green characters in the user table.
- This window allows the creation of new users.
- Users have 2 possible level of access: Users or Administrator (see 7.17.7.1 for more details on different access levels rights)
- Only an Administrator is allowed to create new users.
- Only administrators are allowed to modify setup options except Global.
- A user can only operate the instrument, including addition of lot numbers, changing of kits, and change his own password, change date/time, volume and language.

To create a new user (only accessible from a user currently logged in with ADMIN rights):

1. Click on the **Add New** button



2. Enter the new **User Name**(maximum 20 characters accepted)
3. Select the type of user (**USER or ADMIN**) by clicking on the corresponding radio button
4. Enter and confirm the **password**(maximum 20 characters accepted)
   (Mandatory; if the SAVE button is click with no password an error message will display: *"Password cannot be empty. You need to enter at least one character".*Also, if the User Name already exists, an error message is displayed: *"Please modify the existing or change the name"*)
5. The **SAVE** button will be activated once all information is entered. Click on the **SAVE** button.

Likewise, a user can also be modified: access rights and/or password. But a user with USER rights can only modify his own User limited to his password.

7.17.7.1. Different access rights

There are 4 different access levels in the software: User, Admin, Service and SuperUser. The Service and SuperUser are reserved to special access rights that are not granted to end users. Therefore only user and Admin will be described below.

			User	Admin
Settings	LIS	Modify LIS settings	×	✓
	Assay	Modify assay units (first and second + eAG)	×	✓
		Modify default assay	×	✓
		Modify default test replicates	×	✓
		Modify Calibration mode (automatic/manual)	×	✓
		Modify assay references value	×	✓
		Modify Calibrator replicates	×	✓
	System	Modify system date & time	✓	✓
		Modify sound volume	✓	✓
		Modify system language	✓	✓
	Reports	Add new printer	×	✓
		Modify reports setting	×	✓
	Users	Modify his own password	✓	✓
		Modify other user password	×	✓
		Modify other user rights	×	✓
		Remove a user account	×	✓
		Add a new user account	×	✓
Other	Maintenance	Update the software	×	✓
		Open Adjustment	×	×
		Add records	✓	✓
		Modify components into technical maintenance	✓	×
	Running	Run without a valid cleaning	×	×
		Run without calibration	×	×
		Add Cal or QC lot	✓	✓
		Modify Cal or QC lot	✓	✓
		Accept or reject controls	✓	✓
		Reactivate calibration	×	✓
		Request new calibration	✓	✓
		Request control	✓	✓
		Request cleaning	✓	✓
		Add tube when rack is already loaded	×	×

7.17.8. Special

In this menu, technical information can be retrieved:

- hardware and software versions
- Peak top ranges

8 Maintenance

8.1. Daily

8.1.1. Beginning of day

- **Check that the carousel and the instrument** surfaces are cleaned from any reagent or sample spills *(with Iso Propyl Alcohol (IPA))*
- Check the waste bottle is empty
- If the instrument is on Standby mode, click on the startup icon (See startup 7.4.5). *(Otherwise the system will automatically manage the type of priming needed at the time of the run request).*

8.1.2. End of day

- Perform a cleaning cycle procedure (from Maintenance, Clean, or from the rack positioning).

8.1.2.1. Cleaning procedure

Note: Do not perform Clean and Power OFF procedure. Since instrument is not supposed to be turned OFF. Use only **Comprehensive cleaning option** maintenance menu.

The cleaning procedure is extremely important to keep the system clean and avoid protein build up in the low pressure sections of the system. For this purpose, an enzymatic cleaner solution as well as a rinse solution is used.

There are several ways to request this task:

- By loading the Cleaner and Rinse on the rack
- From the Maintenance menu (2 process of performing it from the maintenance menu):
  - Clean and Power off (overnight soaking, click here to see description)
  - Comprehensive clean (Complete 20 minutes heated clean soaking, click here to see description)
- When placing the system in Standby mode
- When requesting the Power off (if the system is in Ready or Standby mode)

Warning: this is a **mandatory** procedure to be performed minimum 24 hours after the first sample run since the last valid cleaning procedure. It is recommended to run it daily at the end of the last rack. The system will remind the user at each rack ejection to perform it. Once the 24-hour delay is exceeded, **the instrument will not allow running assays until the cleaning is performed**.

Note: The Comprehensive cleaning procedure is programmed to last 5 minutes of contact. During the procedure it is possible to review other things in the software, by clicking anywhere outside of the cleaning window; it will minimize the window and appear as an icon in the bottom menu. Click again on the icon to restore the window normal view

Figure 25: Minimized cleaning procedure, icon located next to the instrument status

8.1.2.1.1. Cleaning procedure from the rack loading

The cleaning procedure can be initiated automatically if the Cleaner and Rinse are positioned on a rack. These 2 solutions must both be present for the cleaning procedure to be initiated. These solutions can be placed either on their own, or at the end of a rack with samples.

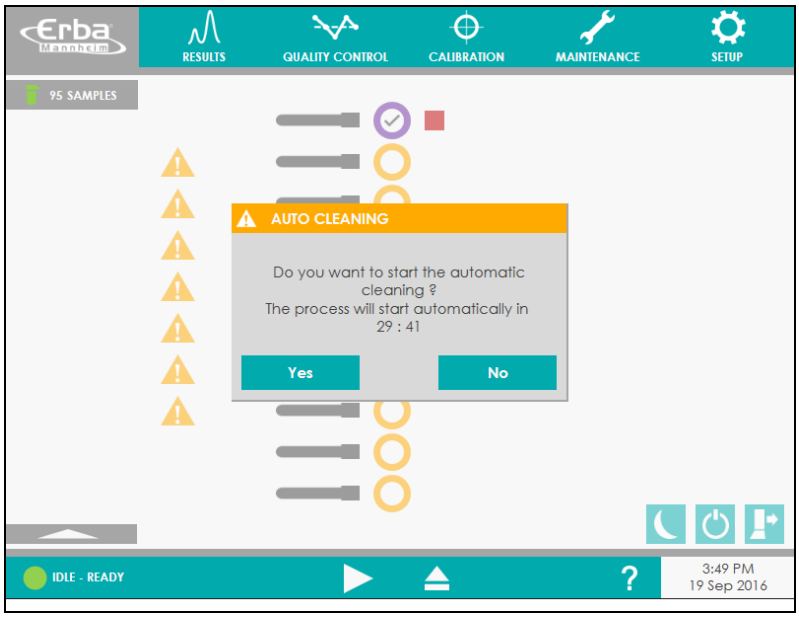
The only possible positions of these 2 solutions are:

- Position 9 = Rinse (in a special 10 ml tube supplied with the kit)
- Position 10 = Cleaner

Warning: The order of positioning of the 2 different cleaning solutions Cleaner and Rinse is extremely important and cannot be reversed, or the **column will be damaged**. Insure that Cleaner is physically positioned on the rack in position 10, and the Rinse in position 9.

Once the 2 solutions are seen on a rack following samples, the system will automatically proceed to the cleaning cycle after reporting the last sample.

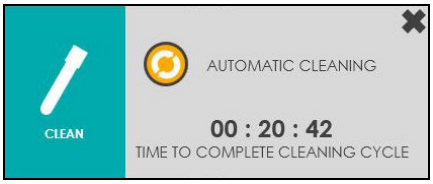
Before proceeding, a message will be displayed: "*Do you want to start the automatic cleaning? The process will start automatically in 30:00*" (Min:sec count down)



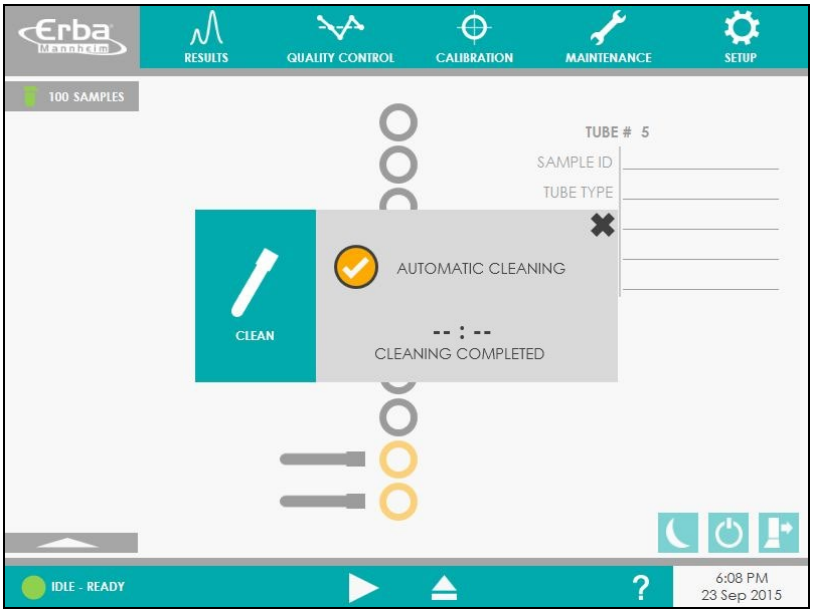
a) Answering No will cancel the procedure, and no cleaning will be performed, allowing the user to eject the rack and regain full access to the system.

b) Answering Yes will start the procedure immediately without the countdown delay.

Once the incubator temperature reaches a temperature of 60°C +/-1°C, the following window will appear.



The process of the cleaning will be exactly as when executed from the maintenance (see Cleaning steps), till its full completion below indicated by the Automatic cleaning checked circle.



Notes: Like the Cleaning procedure initiated from the maintenance menu, the procedure can be interrupted during its process by clicking on the X on the upper part of the cleaning dialogue box. The response of the system to this interruption request is similar to the maintenance Clean interruption.

8.1.2.1.2. Cleaning procedure from the Maintenance Menu

From the maintenance menu the daily cleaning procedure can be achieved in 2 ways:

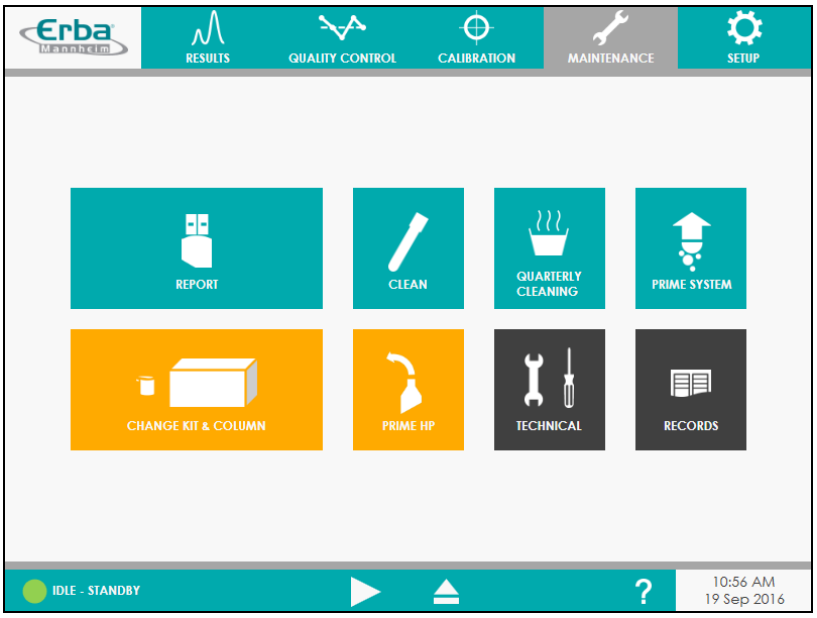
- The Clean and Power off (see 8.1.2.1.2.1)
- The Comprehensive clean (see 8.1.2.1.2.2)

Note: Do not perform Clean and Power OFF procedure. Since instrument is not supposed to be turned OFF. Use only **Comprehensive cleaning option** maintenance menu.

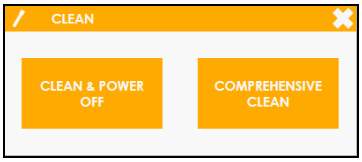
8.1.2.1.2.1. Clean and Power off procedure

To request and perform the daily Clean and Power off procedure from the maintenance menu:

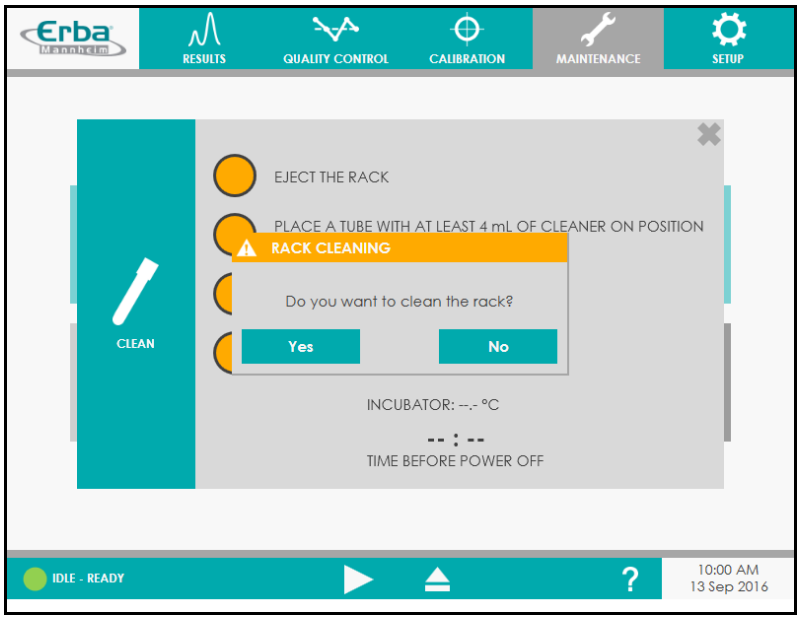
1. Make sure the instrument is in IDLE Ready or Standby mode
2. Click on the Maintenance icon



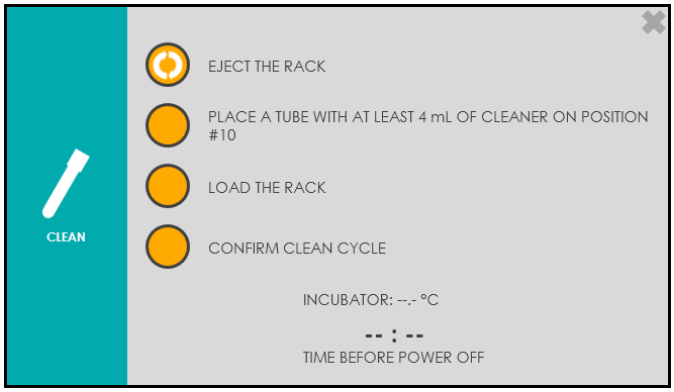
3. Click on the Clean icon
4. The system will display a submenu:



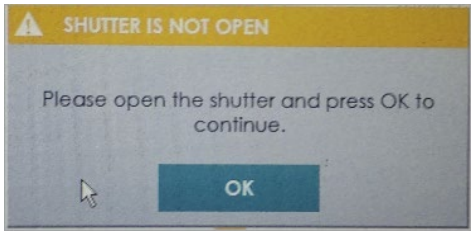
5. Select the CLEAN AND POWER OFF button
6. The system will display a question: “Do you want to clean the rack?”



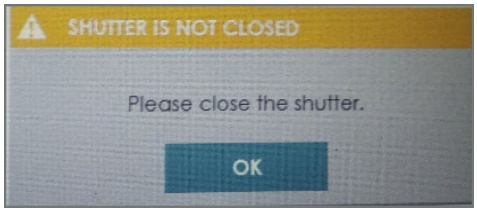
7. Answering No will cancel the procedure, answering Yes will remove all samples defined on board and allow the access to the Clean instructions dialogue box (below)



8. Follow the instructions by clicking on each dot once the step is to be performed by instrument, or once user has completed the step.
   a. Click on Eject the Rack to have the system place the rack holder in the loading/unloading position, *the system will display the revolving arrows before pulling the holder out*
   b. To open the shutter message will get displayed to open the shutter.

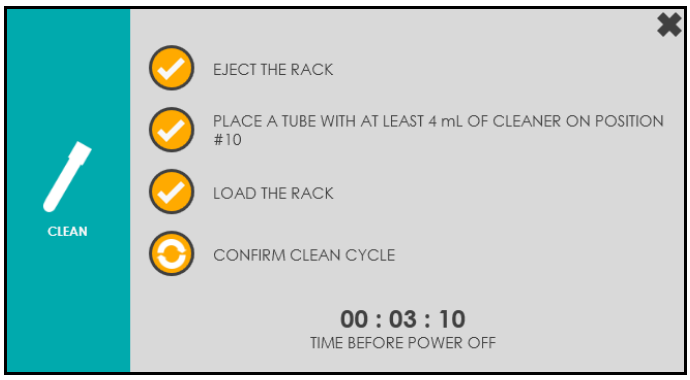


   c. Open the shutter and click OK, sample rack holder will move to the loading/unloading position, ready to accept the sample rack.
   d. After loading /unloading sample rack, message will get displayed to close the shutter.

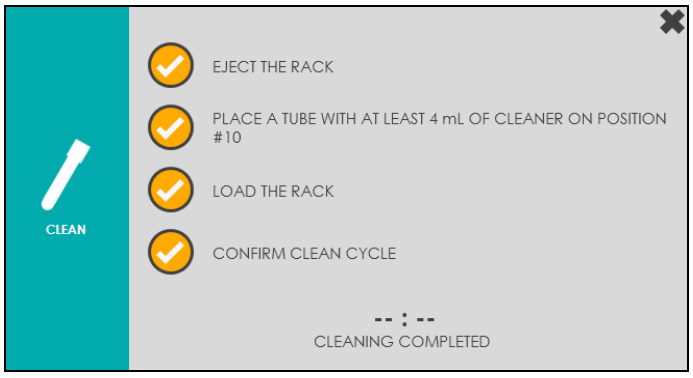


   e. Then click on the following instruction dots once the task is performed

Note: Once all requested tasks have been performed and confirmed, the system will wait to reach the temperature range of 60°C +/-1°C to start the procedure. The temperature will display only during the phase of waiting to reach temperature. Once the temperature range is reached, it will display the time remaining before the Power off takes place in format Hour: Min: Sec.



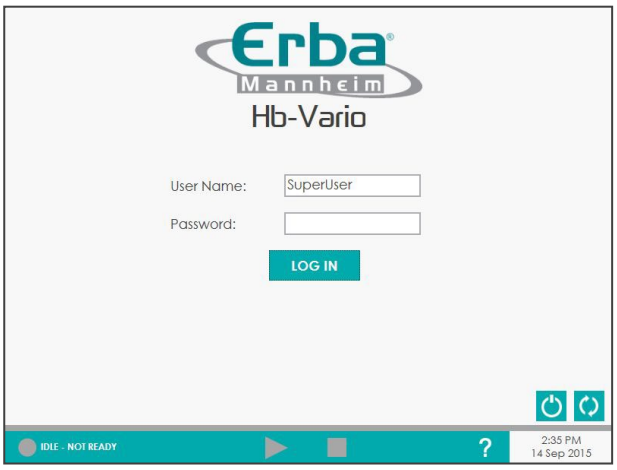
9. After the cleaner aspiration and positioning steps are completed, will display the following window:



10. The system will automatically power off.

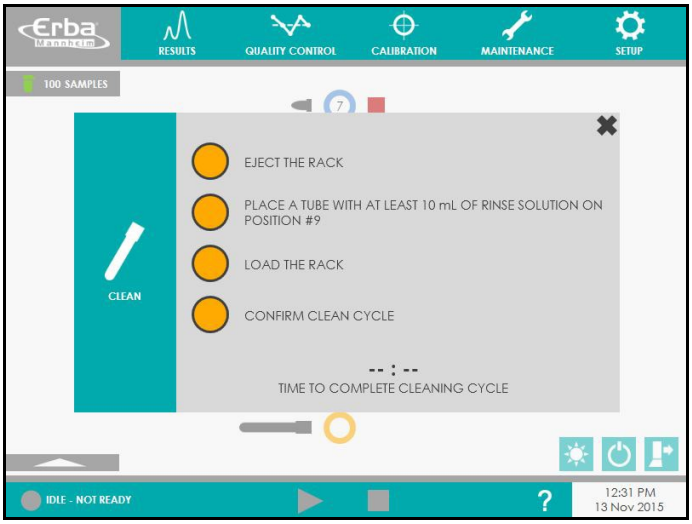
It will beep 5 times during this operation to warn the user that the instrument can be switched off manually afterwards.

11. Once the screen is black, place the power switch on the O position and leave the soaking overnight.
12. Upon return the following morning *(respect the duration prescribed between 8 and 48 hours)*, turn back the instrument ON
13. The system will boot up and load the login page.



14. Enter the user name and password as usual

The system will automatically go to the needed maintenance steps as shown below:



15. Follow the step by step on-screen instructions and click on the required steps to request/confirm, **and answer YES to the question:" Do you want to clean the rack?"**

The system will proceed with the rinsing

16. Then, based on the soaking time performed, the system will either give the completion passing confirmation as follows

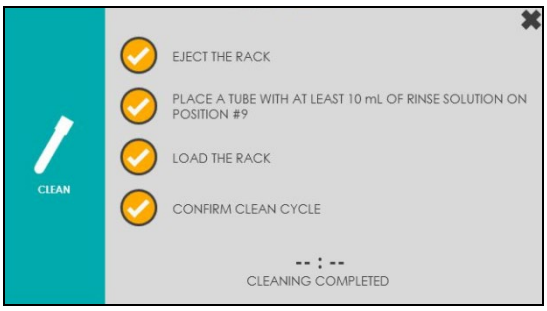


Figure 26: Confirmed successful cleaning procedure, will be registered as valid, countdown will be reinitialized

17. Or will give the completion failure as follows:

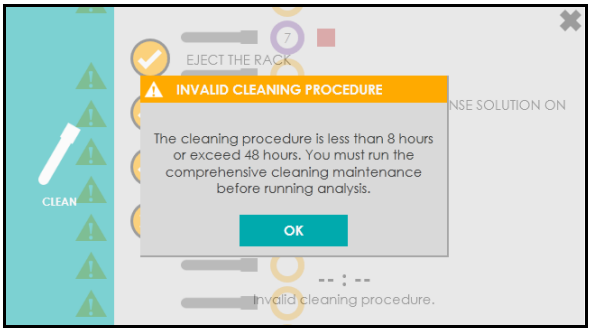
Figure 27: Unsuccessful cleaning procedure, will NOT be registered as valid, countdown will not be reinitialized.

18. Close the window by clicking on the black **X** (top right corner of the window).

WARNING:

The overnight soaking is performed on an instrument in the OFF position. It is therefore not heated; the duration of the soaking has to be sufficient to be effective. It also has to not exceed a maximum duration otherwise it can also generate residues. **Thus the soaking has to be at least 8 hours and less than 48 hours.** If this duration cannot be met, then a comprehensive clean is necessary.

If the cleaning does not meet the minimum and maximum requirements for timing, then the cleaning procedure will not be registered as a valid cleaning, and the 24 hours count down from the first sample run after a successful one continues to run. An error for invalid cleaning procedure is displayed as shown below;



Once the 24 hours are reached, the system will impose an immediate cleaning to be performed.

Description of the clean and power off procedure:

1. The instrument waits till the incubator reaches a temperature of 60°C+/- 1°C and regulates at 60°C
2. The cleaning cycle starts and the incubator is drained
3. The probe aspirates the cleaner from the tube in position 10 and places it into the loop
4. The probe aspirates the cleaner in 4 more successive aspirations, places it in the incubator and leaving 300 µl in the probe for the entire duration of the contact period.
5. The system will power off automatically (beeping for 5 seconds during this phase to warn the user of the process)
6. The user has to manually place the power switch on the O position to complete the power off

Contact will take place overnight (duration of the contact will be evaluated upon the beginning of the rinsing cycle)

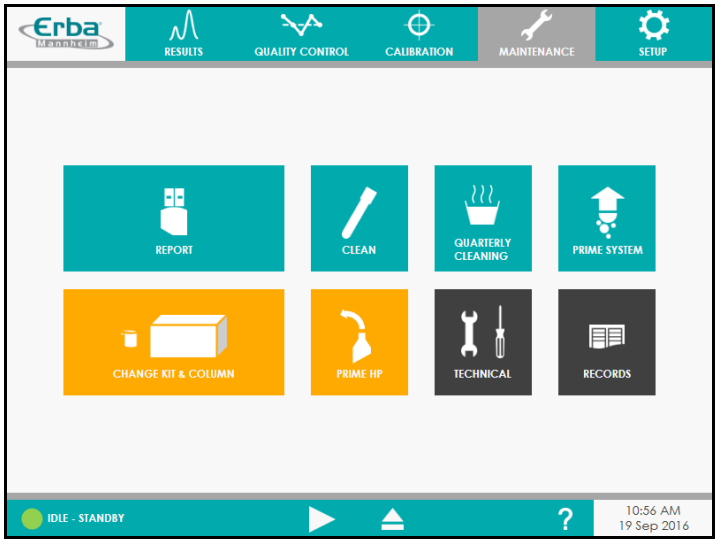
7. The user will power back ON the instrument
8. After Login, the system will automatically display maintenance steps, requesting the rinse tube to be placed on-board
9. The incubator starts heating at 60°C, it is drained and probe emptied and washed
10. The probe immediately aspirates Rinse from tube position 9 and dispenses it in 4 successive aspiration/dispense cycles in the heated incubator
11. The incubator then is drained and Rinse is again aspirated from tube and dispensed in the hot incubator in 4 aspiration/dispense cycles will then rinse the loop

12. Steps 10 and 11 are repeated one more time
13. Then everything is drained and rinsed
14. A message will state whether or not the cleaning procedure is valid depending on the contact duration. (Duration should be between 8 and 48 hours, any duration outside of this range will be deemed unsuccessful and will resume 24 hours count down since the last successful one registered.)

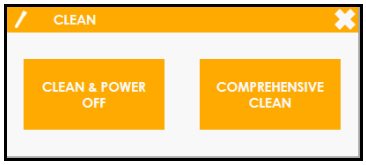
8.1.2.1.2.2. Comprehensive Clean procedure

To request and perform the Comprehensive daily clean procedure from the maintenance menu:

1. Make sure the instrument is in IDLE Ready or Standby mode
2. Click on the Maintenance icon

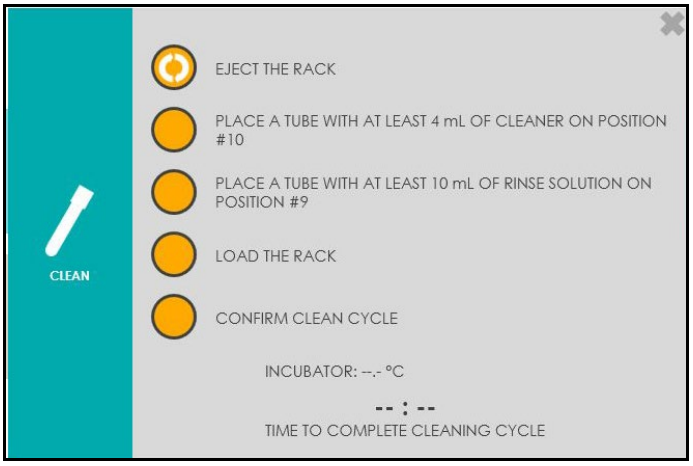


3. Click on the Clean icon
4. The system will display a submenu:



5. Select the COMPREHENSIVE CLEAN button
6. The system will display a question: “Do you want to clean the rack?”

7. Answering No will cancel the procedure, answering Yes will remove all samples defined on board and allow the access to the Clean instructions dialogue box (below)

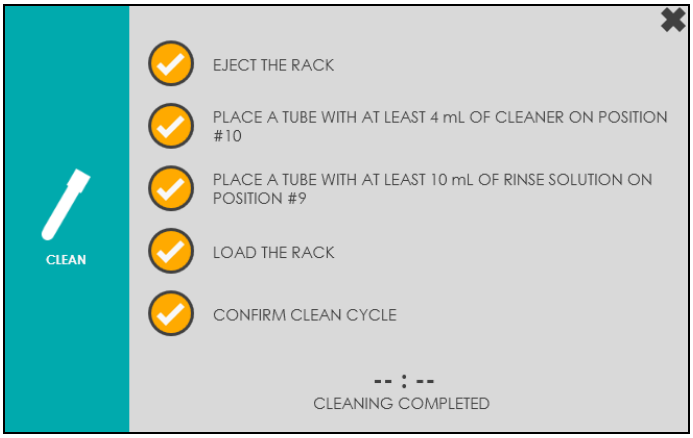


8. Follow the instructions by clicking on each dot once the step is to be performed by instrument, or once user has completed the step.
   a. Click on Eject the Rack to have the system place the rack holder in the loading/unloading position, *the system will display the revolving arrows before pulling the holder out*
   b. Then click on the following instruction dots once the task is performed

Note: Once all requested tasks have been performed and confirmed, the system will wait to reach the temperature range of 60°C +/-1°C to start the procedure. The temperature will display only during the phase of waiting to reach temperature. Once the temperature range is reached, the time remaining before completion of the full procedure will be displayed in format Hour : Min : Sec.

Description of the Comprehensive Clean procedure:

1. The instrument waits till the incubator reaches a temperature of 60°C +/-1°C and regulates at 60°C
2. The cleaning cycle starts and the incubator is drained
3. The probe aspirates the cleaner from the tube in position 10 and places it into the loop
4. The probe aspirates the cleaner in 4 more successive aspirations, places it in the incubator and leaving 300 µl in the probe for the entire duration of the contact period.
5. After the contact period, the incubator is drained and probe emptied and washed
6. The probe aspirates Rinse from tube position 9 and dispenses it in 4 successive aspiration/dispense cycles in the incubator at 60°C
7. The incubator then is drained and Rinse is again aspirated from tube and dispensed in the hot incubator in 4 aspiration/dispense cycles will then rinse the loop
8. Step 6 and 7 are repeated one more time
9. Then everything is drained and rinsed, **ready to run new analyses**
10. When the procedure is completed with the prescribed soaking time, the system confirms the clean cycle with a checked circle and CLEANING COMPLETED confirmation message



Cleaning procedure interruption: The cleaning procedure can be cancelled by pressing the X on the upper right corner of the instructions dialogue box. A message will request the user to confirm the cancelation: “Are you sure to abort the current maintenance?”

- Answering No will continue the procedure and close the question
- Answering Yes will:

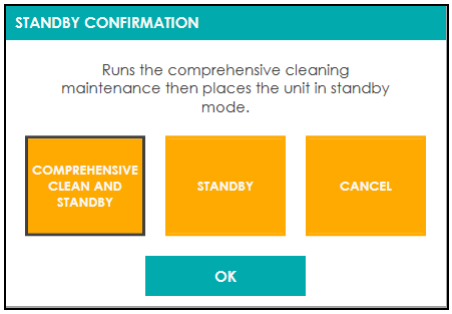
a) In case the cleaner has not yet been aspirated; close the procedure and go back to the main maintenance window
b) In case the cleaner was aspirated, interrupt the contact phase and proceed to Rinse cycles.
c) In both cases, the cleaning cycle will not be registered as successful and will not reinitialize the 24-hour countdown.

8.1.2.1.3. Cleaning procedure from the Standby request

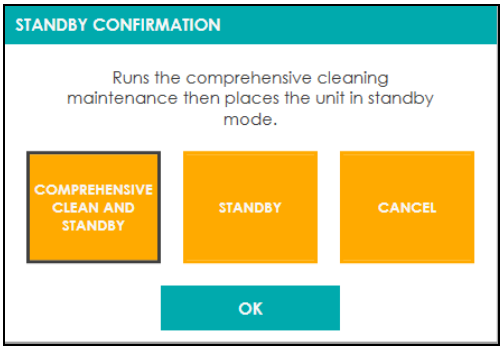
Since the daily cleaning is very important to be performed as soon after the last samples are run, the software will prompt user to perform it when placing the instrument in Standby mode.

Standby is designed as follows:

1. Click on the Moon button from the status window
2. A dialog box will open:

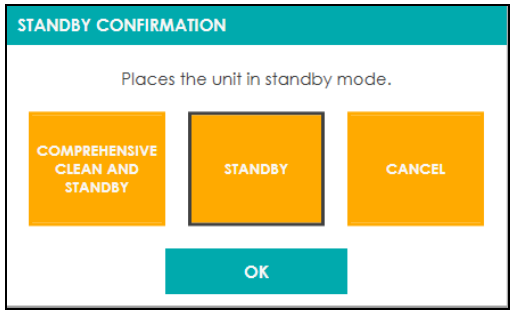


3. It opens by default selections:
   a) "Comprehensive Clean and Standby" if any sample were run since the last effective daily clean recorded.
   b) Otherwise, it will auto select the Standby option.
4. Select the desired option:

a) 

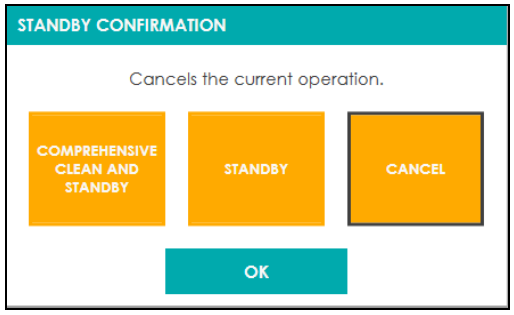
Comprehensive clean and standby

- The system will open the dialog box related to this cleaning cycle. Refer to Comprehensive Clean procedure for details.
- Then it will place the instrument in Standby mode after completion of the clean and rinse cycles.

b) 

Standby

- The instrument will be placed in Standby. The Standby button will toggle to the Startup button to reinitialize the system if additional sample need to be run.

c) 

Cancel

- Cancels the request for Standby, no operation will be done

5. Confirm one of the 3 above choices by clicking on the OK button.

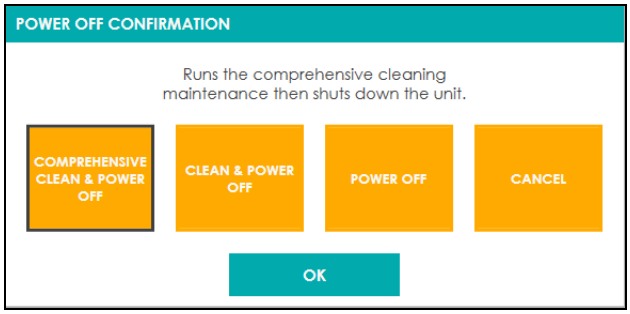
8.1.2.1.4. Cleaning procedure from the Power Off request

Note: Do not perform Clean and Power OFF procedure. Perform Cleaning from maintenance menu. Use only **Comprehensive cleaning option** maintenance menu

Since the daily cleaning is very important to be performed as soon after the last samples are run, the software will prompt user to perform it when requesting to Power Off.

Power Off is designed as follows:

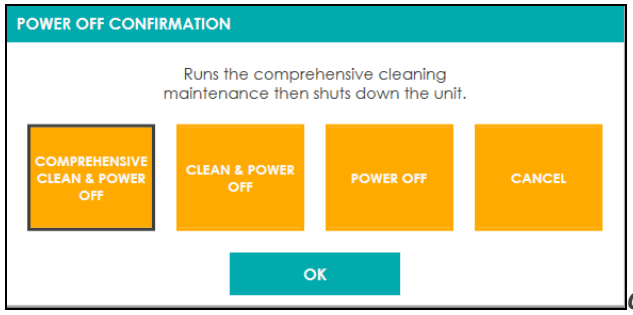
1. Click on the Moon button from the status window
2. A dialog box will open:



3. It opens by default selections:
4. "Comprehensive Clean and Power Off" if any sample were run since the last effective daily clean recorded.

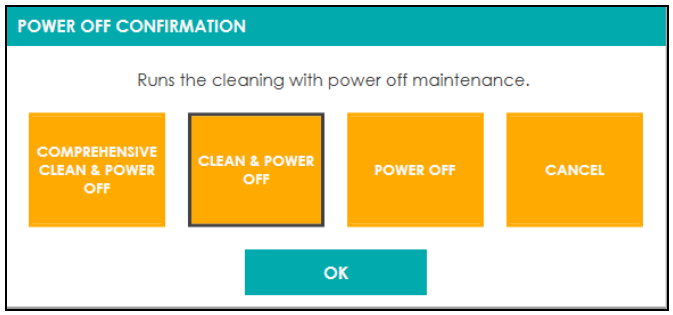
c) Otherwise, it will auto select the Power Off option.

5. Select the desired option:

a) 

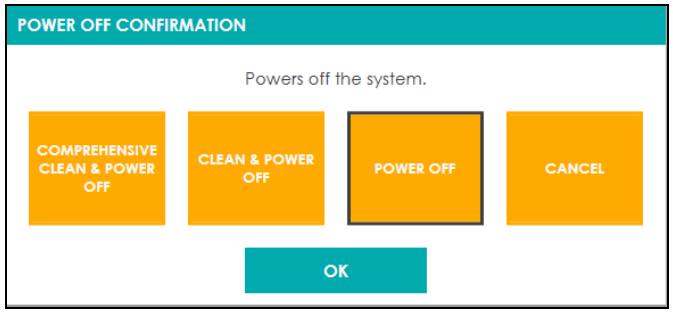
Comprehensive Cleaning and Power Off

- The system will open the dialog box related to this cleaning cycle. Refer to Comprehensive Clean procedure for details.
- Then it will place automatically Power off the software (beeping 5 times before doing so). The user will just need to turn OFF the instrument by placing the switch on the O Position.

b) 

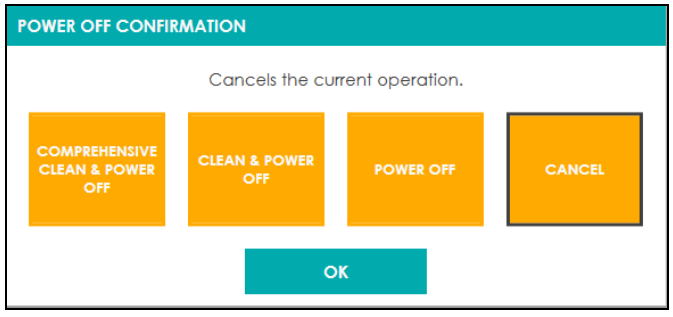
Clean and Power Off

- The system will open the dialog box related to this cleaning cycle. Refer to Clean and Power Off procedure for details.

c) 

Power Off

- The software will powered off immediately without running any cleaning cycle.

d) 

Cancel

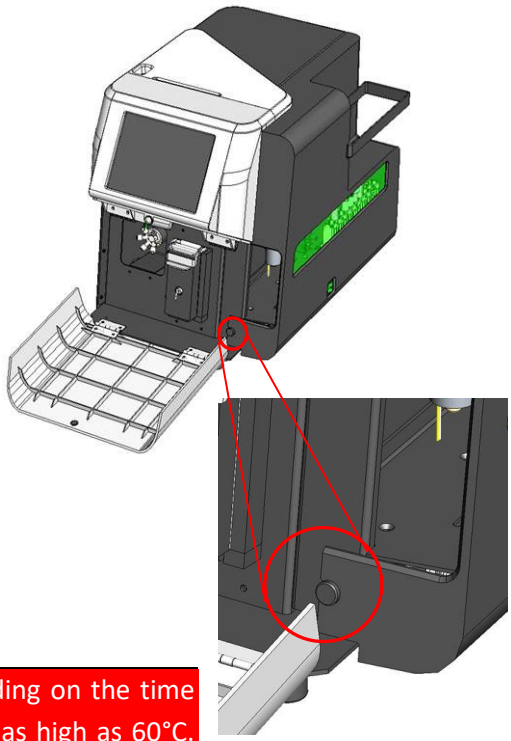
- Cancels the request for Standby, no operation will be done

6. Confirm one of the 4 above choices by clicking on the OK button.

8.2. Weekly

A more complete cleaning of the incubator is necessary on a weekly basis.

For this procedure, it is necessary to prepare a fresh bleach solution in clean distilled or deionized water. The target Active Chloride concentration is 0.9%, which can be obtained by a 1/10 dilution from concentrated liquid bleach or a 1/3 dilution from ready-to-use household bleach.



Warning: Avoid the use of tablet to avoid particles.
If tablets cannot be avoided, filter the solution before use.

1. Do not Switch OFF the instrument
2. Open the right door of the analyzer:
    a. Lower the front panel of the instrument
    b. Loosen the bottom right hand screw
    The upper right hand screw should already be loose enough to allow opening of the right door
    c. Open the right door
3. Move the incubator in a position that allows access

Warning: Be careful to the incubator temperature. Depending on the time elapsed since last used, the incubator temperature can be as high as 60°C. Manipulate it and the liquids extracted from it with caution to avoid injury.

4. Inject around 600µl of the freshly prepared bleach solution into the incubator
5. With a long foam (lint-free) swab, which allows access to the bottom of the incubator, wipe the internal surface of the incubator from bottom up in circular movements in order to get the debris out on the swab. This will detach the potential dried, adherent deposits that the enzymatic cleaner would not be able to remove alone.
6. With a soft Pasteur disposable pipette, aspirate the remaining dirty bleach solution from the incubator.
7. Inject again minimum 600µl of the freshly prepared bleach solution into the incubator
8. Leave the bleach solution in contact for about 10-15 minutes; it will dissolve the remaining protein build-up that may have occurred.
9. During that time, swipe the probe with *Iso Propyl Alcohol*

Warning: Do not leave the bleach solution for more than 30 minutes as it may damage the instrument.
Follow the procedure to the last step.
In case the daily cleaning was performed prior to the weekly procedure, proceed with a startup after the soaking of 15 minutes to remove the bleach from the system, then switch off the instrument.

10. Close back the right analyser door:
    a. guiding the door closed into its 2 up and down loose screws
    b. tighten the bottom screw
    c. reposition the white top paper guide cover
11. After the 10-15 minutes soaking, switch the instrument back ON
12. Then proceed to a regular enzymatic cleaning cycle under the maintenance menu. (see 8.1.2.1.1 Cleaning procedure from the maintenance menu)

Example of equipment required for the weekly maintenance:

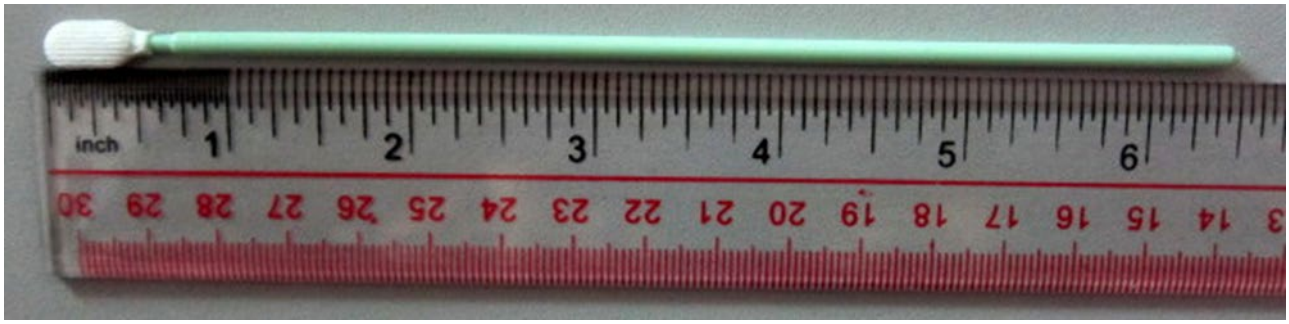


Figure 28: Foam swab

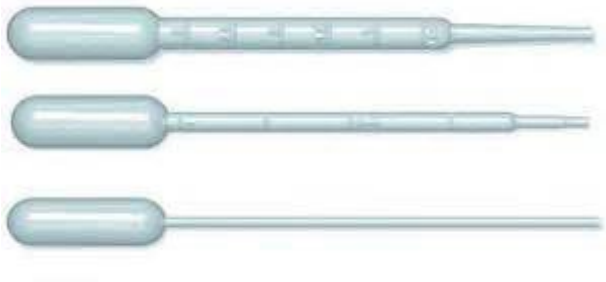


Figure 29: Plastic Pasteur pipettes

8.3. Monthly

There is nothing special to be done on a monthly basis.

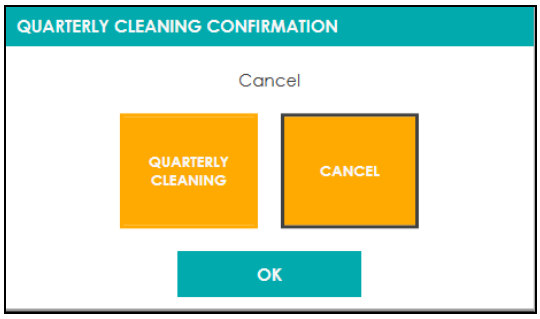
8.4. Quarterly

Every 3 months, a high and low pressure cleaning is needed to maintain the hydraulic system from building up obstructions.

For this Quarterly hydraulic cleaning:

1. Prepare about 250ml of hot water to 45 to 50°C
2. Delicately place the 3 reagent tubings (Hemolyser, Reagent A and Reagent B lines with filters), making sure that no air enter the tubings

3. Select the Quarterly Cleaning from the Maintenance Menu, a selection confirmation popup window will open as shown below, which by security, will be preselected on Cancel



4. Click on OK to confirm Cancellation

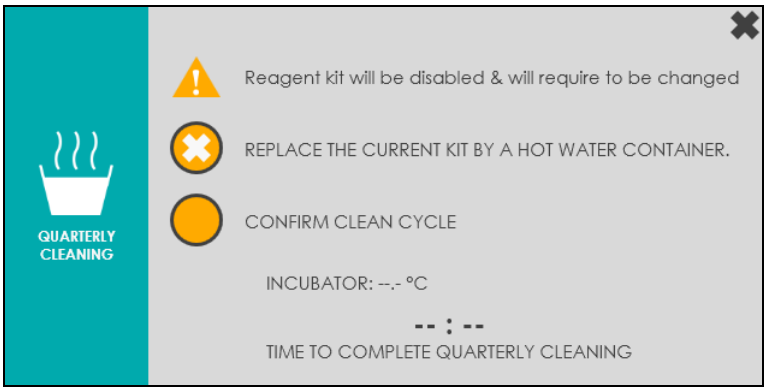
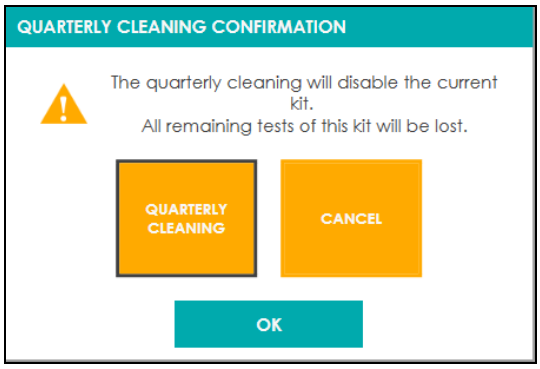


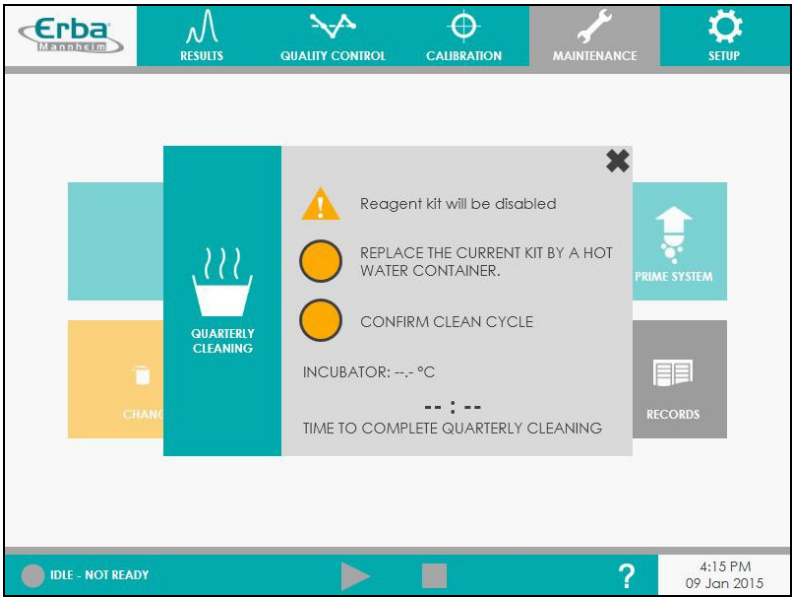
Figure 30: window of quarterly maintenance procedure when Cancel was selected from Popup window

5. To perform the Quarterly Cleaning click on the Quarterly Cleaning button from the selection popup window.

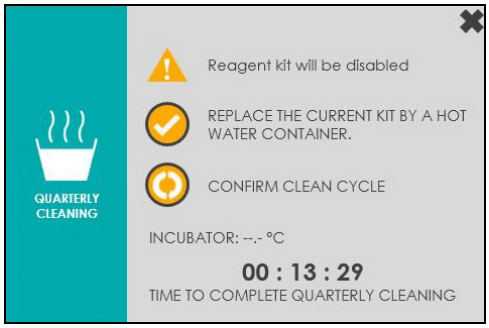


A message informs / reminds that the current kit will be lost if the maintenance cycle is performed.

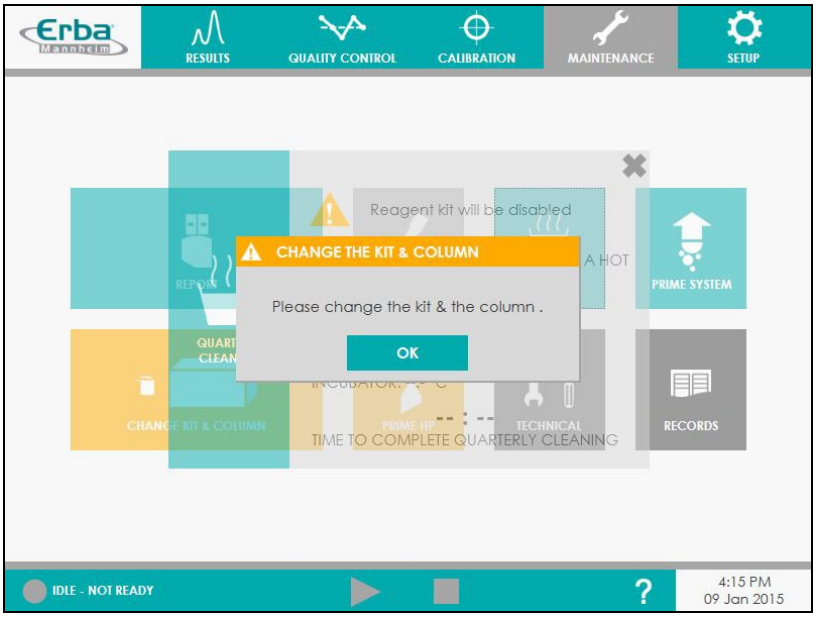
6. Click on OK to confirm



7. Follow the on-screen instructions.



8. Once the cleaning is completed the system will request installation of a new kit.



9. Click OK and proceed to the Change Kit and Column button

Note: the change Kit and Column process in the specific case of following a quarterly Cleaning will take longer as fuller priming will be done to get rid of all traces of water from the lines

Warning: It is recommended to perform the quarterly maintenance on an empty kit (that has just reached the 100 tests). Otherwise the kit started will be invalidated and lost as soon as the first instruction circle will be clicked.

8.5. Biannually

There is nothing special to be done on a 6 months basis.

8.6. Annually

Warning: The annual maintenance procedures are to be performed by a trained and certified Erba technical service personnel or representative.

The annual maintenance consists of:

- The replacement of the peristaltic waste pump tubing
- Replacement of the external and internal evacuation tubings
- Replacement of the Reagent bottles filters (A, B and Hemolyser)
- Replacement of debubblers membrane's (2 Nos)
- Replacement of air filters (2 Nos)
- Replacement of 100µm lysate filter (1 No)
- Measuring and calibrating the flow of priming pump for internal and external probe wash
- Automatic flow rate calibration of evacuation pump
- Auto-adjustment of optical module
- Clean the aperture of the column holder going from column to optics with the same Erba mandrill used to clean the probe every 6 months.

8.7. Before turning off the instrument

In case the instrument must remain unused for a prolonged period, a specific procedure must be followed to insure that the system will function properly when starting the instrument back on, as well as avoiding crystallization and potential damage to the instrument.

- Perform a Quarterly Cleaning procedure using a dummy column, and leave the instrument on water at the end of the cleaning procedure.
- When the cleaning process is complete, Click OK on the message that the kit needs to be replaced.
- Then proceed to the system power off from the Status Window. 

See detailed Quarterly procedure in 8.4

WARNING: Never leave the instrument without a column installed (real or dummy)

9 Troubleshooting

9.1. Diagnostics Chart

Use the following Diagnostics Chart to help diagnose issues with your instrument so that you can report genuine technical service problems in way which will help your technical service team resolve the issue as soon as possible.

Observations	Check the Following
Hb-Vario flags	
Low pressure system is blocked. *This message is present on the test result and generates an emergency stop popup message* *➔ the HbA1c is reported with "*" to be interpreted with caution*	If the message is only one time reported, just repeat the sample to get result without flagging. If the message persists, the liquid no longer goes to the loop: • Check if the incubator is overflowing, if not, check for presences of a leak under the incubator, if present, check that the hydraulic connector is tight or tighten it. • If there is no leak under the incubator and/or it is overflowing, proceed to a rotary valve unblocking procedure • Check if the incubator is still overflowing after the procedure • If unblocking procedure does not solve problem or if presence of a leak under the incubator persist, call for technical assistance
Sample transfer is not good. *Suspicion of improper loop filling* *➔ the HbA1c is reported with "*" to be interpreted with caution*	• Check chromatograms, if total area and results OK, disregard message • If message persists, contact service.
The Sample transfer is too fast. *➔ the HbA1c is reported with "*" to be interpreted with caution*	• If sample was run from a lysate tube, check that the tube is correctly positioned and sufficient lysate is present. • If alarm persists on several samples, contact technical assistance
High concentration sample, total area more than 15000 *The sample total surface is greater than 15000; this alarm is irrelevant on primers* *➔ the HbA1c is reported with "*" to be interpreted with caution*	Check that : • High hemoglobin sample and settled RBC's at bottom of tube. • Inadequate Hemolyzer to sample ratio. • Lysate is high concentrated. • Whole blood sample analyzed in lysate mode. • Rerun the sample as lysate, preparing the lysate dilution with the following volumes: 25µl of sample + 2000µl of Hemolyser

Observations	Check the Following
Low concentration sample. *Low concentration sample, total area less than 2500.* ➔ *the HbA1c is reported with "*" to be interpreted with caution*	Check that : • Whole blood mode selected for lysate sample. • Sample tube / vial cap not removed • Low Hb sample • Check that the sample/vial is physically in the correct position • Check that the sample was not prepared as lysate and run as whole blood • If all above is OK then: • If this is the only flag present on the result: ○ Rerun as lysate preparing the lysate dilution with the following volumes: 75µl of sample + 1000µl of Hemolyser • If presence of other flags like partial clotting: ○ Consult to Sample transfer is too slow / partial clotting in this chart
Sample transfer is too slow / partial clotting. ➔ *the HbA1c is reported with "*" to be interpreted with caution*	• Proceed to a rotary valve unblocking procedure • Check if the incubator is still overflowing after the procedure If unblocking procedure does not solve problem, call for technical assistance
Optical saturation detected ➔ *the HbA1c is NOT reported; only a "*" will be displayed in place of A1c and the instrument is automatically placed in PAUSE*	• Check if Reagent A & B are correctly filling the system by checking the entrance lines and opening the right door and checking the A & B syringes (there should not be bubbles in the syringes) • If the system is not correctly filled in fluids, perform a Prime HP, then a Standby and startup • If the system is correctly filled in fluids, turn instrument OFF and back ON, and perform a startup. • If message appears after start up, contact your local technical support
HbA0 peak not identified within retention time range.	The peak of HbA0 is < 15%; ➔ *No HbA1c can be calculated*
Hba1c peak not identified within retention time range.	Possible cases: HbA1c <2% OR HbA1c not falling in the HbA1c acceptable RT window. Due to low Ta & OR low value sample. Observe chromatogram and subsequent ones: • If the issue is only observed on 1 sample; repeat the sample. • If the issue is also observed on subsequent samples, refer to troubleshooting under the section "others" • If issue cannot be solved by any of the proposed solutions, contact your local technical support
High HbF, HbA1c may be not reliable.	In HbA1c mode, the uncalibrated HbF is >10% ➔ *The HbA1c is reported with "*" to be interpreted with*

Observations	Check the Following
	caution
Abnormal HbF MAY be present - to confirm with further testing.	In HbA1c mode, the uncalibrated HbF is estimated to be > 2.2% if it were calibrated ➔ *The HbA1c is reported normally for estimated HbF 2.2% to 10%* ➔ *Above 10% the HbA1C is flagged "*" to be interpreted with caution*
Abnormal HbA2 MAY be present - to confirm with further testing.	In HbA1c mode, the HbA2 is >5% ➔*The HbA1c is reported normally from 4% to 8%* ➔ *Above 8% the HbA1C is flagged "*" to be interpreted with caution*
Sample may be too old, results may be not reliable. -Recommended fresh EDTA sample	The MetHb peak is > 8% ➔ *The HbA1c is reported with "*" to be interpreted with caution*
Suspected variant @ %t s.	The flag appears if variants eluding on RT windows of the HbC, HbD, HbE or HbS are detected, it states the RT of the variant peak found. ➔ *The HbA1c is reported with "*" to be interpreted with caution*
Suspected homozygote - Further investigation required.	The flag appears if variants are detected on the HbC, HbD, HbE or HbS RT windows, and that their surface is >70% of the total area. ➔ *No HbA1c is reported on homozygote variants*
Total area of unknown peaks more than 8% - HbA1c not reportable.	The total surface of unknown peaks exceeds 8% of the total area. ➔*The HbA1c cannot be safely reported and is therefore not calculated.* Observe chromatogram and subsequent ones: • If the issue is only observed on 1 sample; repeat the sample. • If the issue is also observed on subsequent samples, look for other flags and their respective resolutions • Refer to troubleshooting under the section "others" If issue cannot be solved by any of the proposed solutions, contact your local technical support
Unknown peak area @ %t s - HbA1c not reportable.	An unknown peak located at the given retention time is found to be exceeding the allowable limit for its position. Unknown peak found before A0 with area > 3% or Unknown peak found After A0 with area>6% ➔*The HbA1c cannot be safely reported and is therefore not calculated.* *" Unknown peak found before A0 with area > 3% or Unknown peak found After A0 with area>6%"*

Observations	Check the Following
	Observe chromatogram and subsequent ones: • If the issue is only observed on 1 sample; repeat the sample. • If the issue is also observed on subsequent samples, look for other flags and their respective resolutions • Refer to troubleshooting under the section "others" If issue cannot be solved by any of the proposed solutions, contact your local technical support
HbA2 peak not distinguishable	The chromatogram appears to be invalid by the absence of identification of at least 1 peak needed for the HbA1c calculation. ➔*The HbA1c cannot be calculated and is therefore not reported.* Observe chromatogram and subsequent ones: • If the issue is only observed on 1 sample; repeat the sample. • If the issue is also observed on subsequent samples, look for other flags and their respective resolutions • Refer to troubleshooting under the section "others" If issue cannot be solved by any of the proposed solutions, contact your local technical support
More than 4 unknown peaks - HbA1c not reportable.	The number of unknown peaks exceeds 4; ➔ *The result of HbA1c cannot be safely reported. No result is therefore given for it.* Observe chromatogram and subsequent ones: • If the issue is only observed on 1 sample; repeat the sample. • If the issue is also observed on subsequent samples, look for other flags and their respective resolutions • Refer to troubleshooting under the section "others" If issue cannot be solved by any of the proposed solutions, contact your local technical support
Suspicion of interferences - HbA1c not reportable.	The HbA1c peak detected is too high (exceeding 20%). There is a possible interference creating a positive bias or a coeluting peak in the HbA1c RT. • ➔ *The HbA1c is reported with "*" to be interpreted with caution* Observe chromatogram and subsequent ones: • If the issue is only observed on 1 sample; repeat the sample to verify, if result still >20% seek alternate method for HbA1c. • If the issue is also observed on subsequent samples, look for other flags and their respective resolutions • Refer to troubleshooting under the section "others" • If issue cannot be solved by any of the proposed solutions, contact your local technical support
HbF MAY be too high	In HbA2/HbF assay, the calibrated HbF is >13%

Observations	Check the Following
Abnormal HbA1c MAY be present - to confirm with further testing.	In HbA2/HbF mode, the uncalibrated HbA1C is >5%
HbA1c result cannot be reported due to out of range RT. Please rerun the sample in lysate mode with 2x concentration.	If RT HbAIc ≥ 50, HbA1c & HbA0 peak exists Total area < 8000
HbA1c result cannot be reported due to out of range RT.	RT HbAIc ≥ 50 HbA1c & HbA0 peak exists TA≥8000
Hb-Vario Popup windows	
Primer is on low concentration. Calibration is aborted, please check calibrator volumes and restart. If the problem persists, please contact your local technical support.	Total area of primer <6000 • Insure that the calibrators are placed directly in a sample vial and not diluted as lysates • Insure that there is sufficient level of calibrator 2 (220µl) • Check that calibrators were correctly reconstituted. • Check the results to look for other flags and consult this troubleshooting chart • Check the total areas of other calibrators, if abnormally low, Consult low concentration sample from this chart
HbA1c calibration has failed for the following reason(s): X a) HbA0 peak not found on primer-RT test b) HbA0 area is too low on primer-RT test c) HbA1c peak not found on primer-RT test. d) The sample area is too low on primer-RT test. e) Improper blank detected	1 or more of these reasons will reject the calibration • Physically verify the onboard level 1 calibrator vial • Verify that the Level 1 calibrator was placed on-board as a whole blood sample and not diluted • Check that the level 1 calibrator was reconstituted with the correct 0.5ml volume • Check Primer RT chromatogram as well as those of primers to check peaks positions and presence of flags. • If the error is on the blank, rerun a calibration to repeat the blank.
HbA1c calibration has failed for the following reason(s): X No HbA1c detected into minimum 1 test of calibrator level 1.	The calibration was rejected: • **HbA0 peak was no found** • **HbA1c was too low** • Physically verify the onboard level 1 calibrator vial • Verify that the Level 1 calibrator was placed on-board as a whole blood sample and not diluted • Check that the level 1 calibrator was reconstituted with the correct 0.5ml volume • Check the chromatograms as well as those of primers to check peaks positions and presence of flags
HbA1c calibration has failed for the following reason(s): X No HbA1c detected into minimum 1 test of calibrator level 2.	The calibration was rejected: • **HbA0 peak was no found** • **HbA1c was too low** • Physically verify the onboard level 2 calibrator vial • Verify that the Level 2 calibrator was placed on-board as a

Observations	Check the Following
	whole blood sample and not diluted • Check that the level 2 calibrator was reconstituted with the correct 0.5ml volume • Check the chromatograms as well as those of primers to check peaks positions and presence of flags
HbA1c calibration has failed for the following reason(s): X a) Calibration factor is out of range (0.97 - 1.25): X b) Calibration Offset is out of range (-0.8 – 1.00): X c) There are more than 6% of variation between high level calibrators: X d) There are more than 6% of variation between low level calibrators: X e) Low level HbA1c RT is out of range. (45.5-49.5): X f) One or more primer has a low concentration flag. Ensure enough L2 calibrator in vial and re-calibrate. g) R^2 is too low (<0.99): X h) Column temperature has been adjusted from X to Y. i) Column temperature adjustment not possible. Contact your local technical support. j) At least one calibrator is in low concentration k) At least one calibrator is in high concentration	1 or more of these reasons will reject the calibration a) and b)**Factor and Offset**: i) Check that the values entered for both levels of calibrators are correct for the lot used. ii) Check that all reagent lines are correctly inserted in the correct reagents (Hemolyser, Reagent A and B) c) **Variation** of High calibrator: i) Check that the calibrator was freshly reconstituted before calibration ii) Repeat calibration procedure d) Variation of Low calibrator (see point b related to the low calibrator) e) Retention Times of Low calibrators: (see point c related to low calibrator) f) **Low Concentration of primers**: (total area <6000) i) Insure that the calibrators are placed directly in a sample vial and not diluted as lysates ii) Insure that they are positioned properly into the rack (sitting correctly into their adaptors) iii) Insure that there is sufficient level of calibrator 2 iv) Check that calibrators were correctly reconstituted. v) Check that there is no leakage around the column holder vi) Check the results to look for other flags vii) Check the total areas of other calibrators, if abnormally low, Consult low concentration sample from this chart g) **R^2 too low**:(the alignment of the replicated calibrators on the linear fitting is < 0.99) i) Check that the calibrators are correctly positioned. ii) Check the lot number actually used is matching the one defined in the software. iii) Check that the values entered for both levels of calibrators are correct for the lot used. iv) Check that calibrators were correctly reconstituted. h) **Column temperature has been adjusted from X to Y**:(An automatic column adjustment was needed and has been set and will be applied for the next calibration procedure) i) **Column temperature adjustment not possible**: i) Contact your local technical support to assist you with troubleshooting specifics of this alarm. j) **One calibrator low concentration** (TA <2500) i) Insure that the calibrator is placed directly in a sample vial and not diluted as lysates ii) Insure that it is positioned properly into the rack (sitting correctly into its adaptor) iii) Insure that there is sufficient level of the calibrator

Observations	Check the Following
	iv) Check that the calibrator is correctly reconstituted. v) Check that there is no leakage around the column holder vi) Check the results to look for other flags vii) Check the total areas of other calibrators, if abnormally low, Consult low concentration sample from this chart k) **One calibrator high concentration** (total area >15000) i) Insure that the calibrator is positioned properly into the rack (sitting correctly into its adaptor) ii) Check that the calibrator was prepared according to its mode (lysate if lysate and not placed pure for a lysate mode) iii) Check that the calibrator is correctly reconstituted. **Once all points are checked proceed to a new calibration procedure.**
HbA1c CALIBRATION FAILED. Please refer to troubleshooting guide or contact your local technical support.	• Refer to all resolution proposals for calibration failure • Once all is checked and cleared, contact your local technical support to assist you with the specifics of the current behaviors.
The calibration failed for the third time. Please change the kit, before requesting a new calibration, or contact your local technical support.	The new kit failed 3 calibrators in a row, it is recommended to change the kit. • Observe chromatograms of the different calibrators and the different reasons given for failures • Contact your local technical support with the information to see if they can assist you with resolution steps. Note: Additional calibrations are still possible on the same kit but will be counted down from the 100 tests for the kit.
The kit calibration failed for the third time, on 2 kits. Please contact your local technical support.	2 kits in a row failed 3 calibrators each. • Contact your local technical support
3 consecutive invalid chromatograms detected. There may be a problem with the system. Please contact your local technical support.	This message will appear in HbA1c mode when the software detects3 consecutive impossibilities to report HbA1c. • Refer to all solutions linked with flags reported on the chromatograms • Refer to the troubleshooting chart section “others” for additional verifications • Click OK to close the message *The count will be reset. The message will only reappear after 3 new consecutive invalid chromatograms.* • Contact your local technical support if the problem persists
Reagent kit will be disabled & will require to be changed	This message will appear when the kit will be damaged by the requested operation. It is therefore very important to understand that the current kit and the column will no longer be usable, and that any remaining tests of that kit will be lost.

Observations	Check the Following
	➔If the operation can be postponed till the end of the kit, it is recommended to wait and perform it then.
The kit has been changed. Please perform the 'Change Kit' procedure and restart.	Proceed to the Change kit procedure *(see 7.16.5 change kit, and 4.3 Installing reagents)*
The kit is not found. Please check if the door is closed and the kit is present, then restart.	• Check that the right instrument door is properly closed • Check that the kit RFID label is present on the kit facing inside the instrument *If loss of RFID communication occurs during the run, the system will automatically pause*
The Hemolyser bottle is empty. Please change it then press 'OK'.	Replace the depleted Hemolyser container from the kit with a new and full Hemolyser bottle (**Hb-Vario Hemolyser Reagent, Reference REG00042** 1 x 900ml)
Kit is finished, please change the kit and column or perform a clean	This message appears when the 100 tests have been reached. The remaining volume is sufficient to perform a clean before anything else. • Replace the kit to resume sample testing
Cleaning will not be available if the system has to start up again, until a new kit is installed (not enough reagents).	This message appears due to low remaining reagent volumes • When after testing the remaining volume is just enough to perform the cleaning, but not enough to allow to go to standby or power off before doing so. • Or in case of refusal of the auto clean cycle ➔ It is recommended to perform the cleaning before Standby or Power off. Otherwise, the cleaning will require a kit replacement to be performed.
The cleaning procedure exceeds 48 hours. You must run the comprehensive cleaning maintenance before running analysis.	This message appears if the system has performed the first part of the Clean and Power Off procedure till Power OFF, and the rinse has been delayed by more than 48 hours. A comprehensive cleaning is needed to restore system operations. Refer to 8.1.2.1.2.2 for details on performing the comprehensive cleaning.
Not enough tests left to run all the worklist. Only X samples can be run:Y	There is not enough of at least one component: Review section 6.11.2 of this manual for instructions
Empty/Unknown positions (X) X represents the number of Empty and/or unknown positions Empty= No barcode seen and no magnetic adaptor Unknown= Magnetic adaptor detected, but no barcode seen and no user entered information for the position	If the X does not match the real conditions: **If barcodes are not read correctly**: • Check that the barcode labels are correctly positioned on the tubes • Check that the barcode labels/prints are of good quality • Check that the barcode labels are correctly oriented towards the barcode reader • With instrument switched OFF, clean the barcode reader • Possible problem with barcode reader, contact your technical service representative ***If sample barcodes are not detected at the correct instrument position:*** • Check that there is no physical obstacle on the sample rack

Observations	Check the Following
	rail. • Contact your technical service representative **For unknown samples:** • Enter relevant information/request in the correct position ***If adaptor is detected at an incorrect position:*** • Check that there is no physical obstacle on the sample rack rail. • Contact your technical service representative
Mismatch ID will not be run. There is a conflict between the manual entry and the automatic barcode reading	• Click on the sample to make the decision • If this occurs repeatedly unjustifiably, consult unknown position message from this chart
"There is no communication with boards. System is in Offline mode."	• Perform a proper full system power off. • Restart the system. • If problem persists, contact your local technical service
The pipette version is not set.	• Perform a proper full system power off. • Restart the system. • If problem persists, contact your local technical service
The hydraulic version is not set.	• Perform a proper full system power off. • Restart the system. • If problem persists, contact your local technical service
The incubator version is not set.	• Perform a proper full system power off. • Restart the system. • If problem persists, contact your local technical service
The 1 mL pump version is not set.	• Perform a proper full system power off. • Restart the system. • If problem persists, contact your local technical service
EMERGENCY STOP - Rack (x) Home sensor problem EMERGENCY STOP - Pippete (x) Home sensor problem EMERGENCY STOP - Sample syringe (x) Home sensor problem EMERGENCY STOP - Incubator (x) Home sensor problem EMERGENCY STOP - Syringe A (x) Home sensor problem EMERGENCY STOP - Syringe B (x) Home sensor problem EMERGENCY STOP - Rotary valve (x) Home sensor problem	• Perform a proper full system power off. • Restart the system and perform a Startup. • If problem is not solved by Startup, contact service, specify the Home sensor mentioned in the error message
EMERGENCY STOP Pressure failure " Clot detected in sample path"	This message will result in an immediate instrument STOP Most probable cause is a loop blockage • Perform a proper full system power off. • Restart the system and perform a Startup. • If problem is not solved by Startup, proceed to a rotary valve unblocking procedure

Observations	Check the Following
EMERGENCY STOP - Evacuation issue The evacuation process seems to not be complete	This message appears when the incubator does not drain completely; it will lead to a stop after completion of the sample already injected in the column. • Turn OFF instrument, and turn it back ON • Perform a startup cycle • If message appears during start up, contact your local technical service
A stop has been raised by the software due to exception. " Recommend to restart the system"	An immediate emergency stop is generated. • Perform a proper full system power off. • Restart the system and perform a Startup. • If problem is not solved by Startup, contact your local technical support
Are you sure you want to stop the run? All in progress tests will be cancelled.	• Stop the run • In progress test will be cancelled
Sample transfer cannot be adjusted, please contact your local technical support	This message can occur; it is still possible to run tests. • If the message occurs every 3 tests: • Perform a rotary valve unblocking procedure to eliminate a partial blockage • If the message persists after that procedure, report it to your local technical support
Optical detector gain cannot be adjusted, please contact your local technical support.	The optical detector displays out of range measurements during startup process. The instrument can be run. Optical saturation is constantly monitored during the run and if detected would not reported HbA1c and the instrument would be paused automatically *(see Optical saturation flag for more details)* • Contact your local technical support to report the alarm for their next visit.
Optical detector gain adjustment limit reached, technical support needs to be planed to maintain working system	The optical detector displays out of range measurements during startup process. The instrument can be run. Optical saturation is constantly monitored during the run and if detected would not reported HbA1c and the instrument would be paused automatically *(see Optical saturation flag for more details)* • Contact your local technical support to report the alarm for their next visit.
Optical saturation has been detected, please contact your local technical support	This message can appear during startup or during a run. • Check if Reagent A & B are correctly filling the system by checking the entrance lines and opening the right door and checking the A & B syringes (there should not be bubbles in the syringes) • If the system is not correctly filled in fluids, perform a Prime HP, then a Standby and startup • If the system is correctly filled in fluids, turn instrument OFF and back ON, and perform a startup. If message appears after start up, contact your local technical

Observations	Check the Following
	support
COLUMN PELTIER PROBLEM Column cooling does not seem to be working correctly, please contact your local technical support.	• Contact your local technical support
COLUMN TEMPERATURE PROBLEM Column temperature regulation does not seem to be working correctly, please contact your local technical support.	• Contact your local technical support
INCUBATOR HEATER PROBLEM Incubator heater does not seem to be working correctly, please contact your local technical support.	• Contact your local technical support
COLUMN & INCUBATOR TEMPERATURE PROBLEM Temperature regulation for the incubator and the column does not seem to be working correctly, please contact your local technical support.	• Contact your local technical support
Some parts need to be updated: X Problem of automatic upgrade failure And/or Instrument upgrade is required	• Contact your local service representative
	•
RT a1c ≥ 50 HbA1c & HbA0 peak exists	For 3 consecutive Kit T° can still be adjusted (< 0.8°C) • New calibration is recommended Kit T° cannot be adjusted anymore (= 0.8°C) • Please contact your local technical service.
Maintenance steps messages	
Invalid kit detected RFID error: Unknown coding or mismatched with instrument configuration	• If possible, try another kit (from another box) • If problem persists, contact your local technical support
The kit is not valid for this unit. RFID related error: Mismatched	• If possible try another kit (from another box) • If problem persists, contact your local technical support

Observations	Check the Following
coding with instrument configuration	
No kit detected RFID related error: the tag could not be read	• If possible try another reagent pack • If problem persists, contact your local technical support
The tube cannot be changed when the unit is running. Please wait till the schedule is completed or click pause.	• Complete the schedule and change the tube
Others	
Magnetic adaptors are not detected	• Check that the adaptor(s) still have their magnets attached • Possible defective magnetic sensor
System does not add automatically default analysis and replicate to tubes automatically detected by barcode reader	• Check that the system does not have the LIS activated by mistake. • Refer to the 10.5 LIS/System Configuration section for more information
Completely flat lines instead of chromatography. ***Note:*** *This means no bumps or changes in the line are visible at all*	• Insure that there is no obvious leakage from the column holder • Report this issue to your technical service team as a *'Possible Detector Issue'*
Either very low signal or an almost completely flat line with small bumps or changes visible.	• Check that there is sufficient sample in the tube / vial you are sampling from. If not, consider preparing a pre-dilute sample. • If sample is sufficient, check that the instrument is actually aspirating blood from the tubes. • Check that the incubator is not overflowing. • Check that there are sufficient reagents remaining in the kit you are using, if not Replace the kit *(see 7.16.5 change kit, and 4.3 Installing reagents)* • Check for leakage around the column holder and/or at the loop
Incorrect results associated with base line shift.	• Check that the instrument is operated within temperature specifications (17 to 32°C) • Check for any step like behavior in the chromatogram tail • If presence of step, contact your local technical support for assistance in identifying check valve leakage.
Abnormal chromatogram with a large peak combining HbA1c to HbA0	Possible inversion of Hemolyser and Reagent A. • Proceed to reinstalling reagents correctly • Prime HP with lines correctly reinstalled • *Run system with a critical eye, it is likely that the kit was contaminated and will require to be discarded and replaced by a new one)*
A single very large, very tall peak at the very beginning of the assay	Likely, that reagents A and B have been installed in the reverse order.

Observations	Check the Following
	• Proceed to reinstalling reagents correctly • Prime HP with lines correctly reinstalled • *Run system with a critical eye, it is likely that the kit was contaminated and will require to be discarded and replaced by a new one)*
All peaks shifted to the left. Possible that some peaks are no longer within designated retention times	This suggests increased reagent flow, possible check valve blockage (even partial), high column temperature or possible reagent contamination. • Check the Column temperature using the Gauges Button on the Status Window (*See **7.6.2**)*. If the Gauges Display is automatically opened by the system, check that it is not due to the column temperature. • Observe the high pressure pumps for visible bubbles, if present, Perform a High Pressure Pump Prime HP (High Pressure Priming), perform it with the right door open
All peaks shifted to the right. Possible that some peaks are no longer within designated retention times	This suggests low reagent flow, possible check valve blockage (even partial), low column temperature or possible reagent dilution or contamination or leakage in high pressure • Check the Column temperature using the Gauges Button on the Status Window (*See **7.6.2**)*. If the Gauges Display is automatically opened by the system, check that it is not due to the column temperature. • From the results window, as soon as a new chromatogram starts displaying, remove the tubings from the A and B lines, and insure no drops are coming out of the tubings after wiping. • Perform *a High Pressure Pump Priming* • Check the underside of the instrument for fluid leaks.

9.2. User resolution procedures

9.2.1. Barcode reader cleaning

If the barcode reader requires cleaning, proceed as follows:

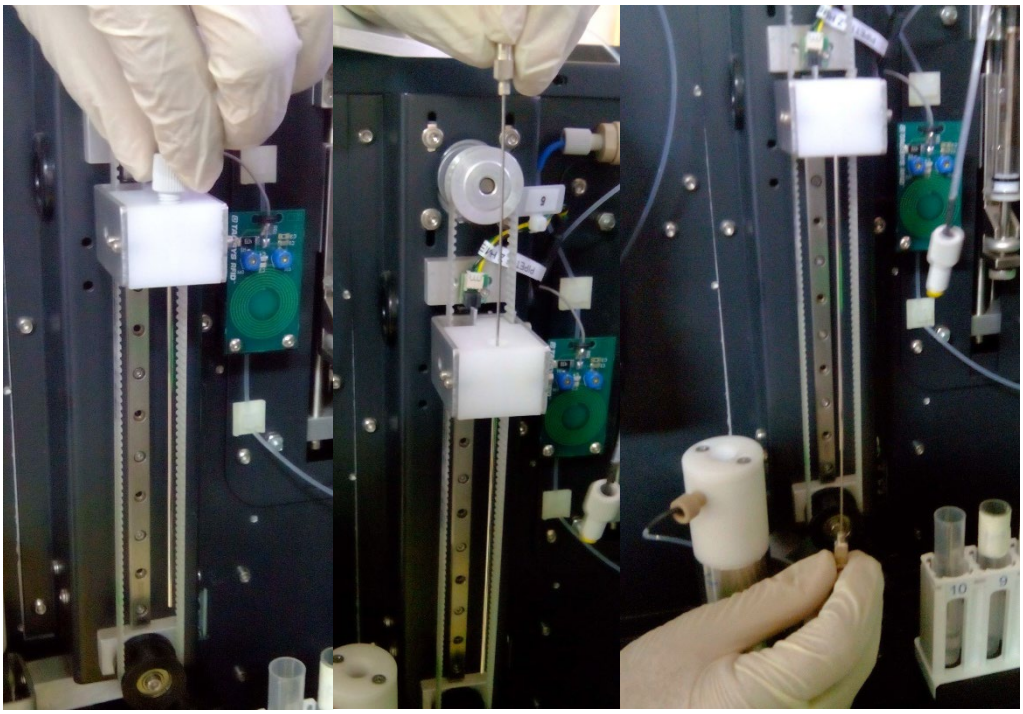
1. Turn OFF instrument 2. Open the right instrument door 3. Locate the barcode reader next to the laser caution label	
4. Barely moisten a cotton swab 5. Delicately clean the 2 square shapes that emit the laser beams 6. Delicately clean the central round shape reader 7. Then take a clean dry swab and dry all cleaned surfaces 8. Close back the right door and resume operations	

9.2.2. Unblocking probe procedure

If the probe is plugged and the probe can be unblocked be cleaned by a mandril

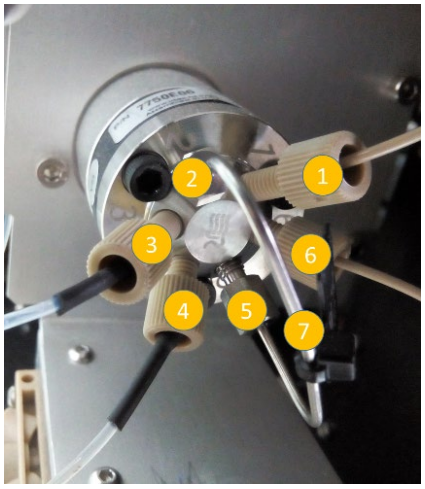
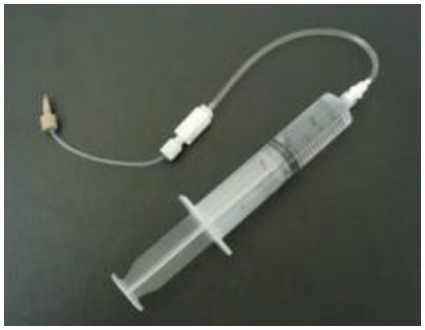
- Use the specific Erba part as shown below:

- Turn OFF the instrument
- The top hydraulic connector of the probe should be disconnected
- Then the mandril will be inserted from the top and then the bottom of the probe in order to remove any partial build ups.



9.2.3. Rotary valve unblocking procedure

Removing a blockage in the rotary valve in the low pressure side

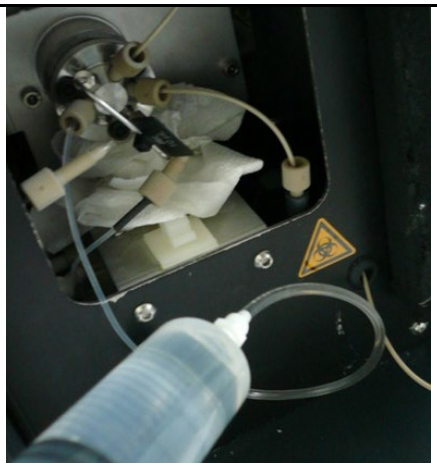
1. Open the instrument front access door	
2. Install an absorbent paper under the rotary valve 3. Unscrew the inlet and outlet like displayed on the picture (connectors 3 and 4)	
4. Aspirate hot water in a syringe adapted with fittings compatible with the rotary valve. (The mounting on the picture is an example, other possibilities can be furnished in the package)	

5. Push the water through the valve the outlet (n°4) and the inlet (n°3).

6. Put the inlet connector back in place and purge again to rinse the sample line.

7. Put the outlet connector back into place

8. Remove the absorbing paper.

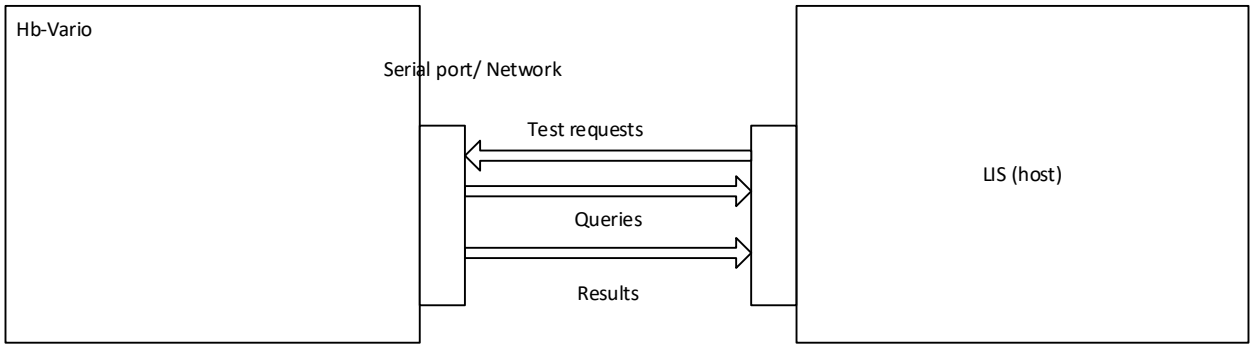


10 LIS Setup

10.1.General

The Hb-Vario System is linkable to a LIS (Laboratory Information System). For that you will need to:

- Physically connect the Hb-Vario to the laboratory system
- Configure the Hb-Vario

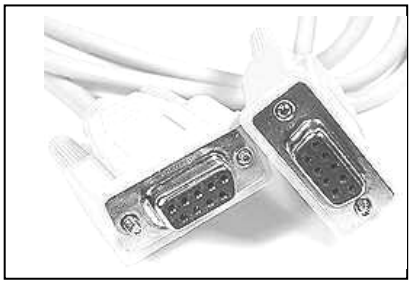


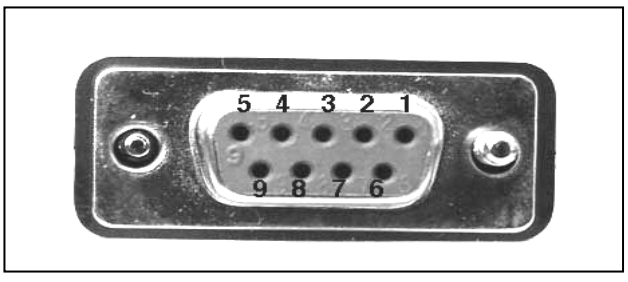
Connection may be done either by serial port or network connection.

10.2.Hardware configuration

10.2.1. Serial communication

The connection uses a **RS232 serial interface** with DB-9 connector between the Hb-Vario and the Host computer.





RS232 Pin Assignments (DB9 PC signal set)	
Pin 1	Received Line Signal Detector (Data Carrier Detect) (DCD)
Pin 2	Received Data (RD)
Pin 3	Transmit Data (TD)
Pin 4	Data Terminal Ready (DTR)
Pin 5	Signal Ground
Pin 6	Data Set Ready (DSR)
Pin 7	Request To Send (RTS)
Pin 8	Clear To Send (CTS)
Pin 9	Ring Indicator

Data:
Low level:
+5 ⇒ +20 V
High level:
-5 ⇒ -20 V
Control:
OFF:
+5 ⇒ +20 V
ON:
-5 ⇒ -20 V

The serial cable must be plugged to the standard DB9 connectors of the Hb-Vario.

10.2.2. TCP-IP communication

The network connection is done by plugging an Ethernet RJ 45 Category 5 cable connector at the rear of Hb-Vario.

10.3. Work modes

2 modes of functioning are possible: Download or Query.

10.3.1. “Download” mode

In this mode, the LIS sends all the requests for the analyser. The Hb-Vario delays the analysis for samples that are not on board. It executes possible analysis then sends the results.

10.3.2. “Query” mode

Once a sample has been identified on the Hb-Vario (with the internal barcode reader, with a handy barcode reader, or with manual test request), the system sends to the LIS a Query for the sample. Then, the LIS sends only the requests concerning this sample.

Once tests are executed, results are sent by the Hb-Vario.

Notice: Hb-Vario doesn’t check adequacy between queries sent and request received, and does not re-query automatically (to ask again requests for tube where no analysis has been received, for example)): so, **rerun** requested from the LIS must be sent directly by the LIS without waiting a new query.

10.4.Protocols

When using serial port, the Hb-Vario works with 2 standard protocols:

- ASTM 1381 for « physical » communication: this protocol describes the mechanisms of data send.
- ASTM E 1394 for « logical » communication: this protocol describes the mechanism of data coding (test requests, queries, results)

Note: With TCP-IP communication, only ASTM E 1394 protocol is used: the physical protocol is TCP-IP.

10.4.1. Physical protocol: ASTM 1381

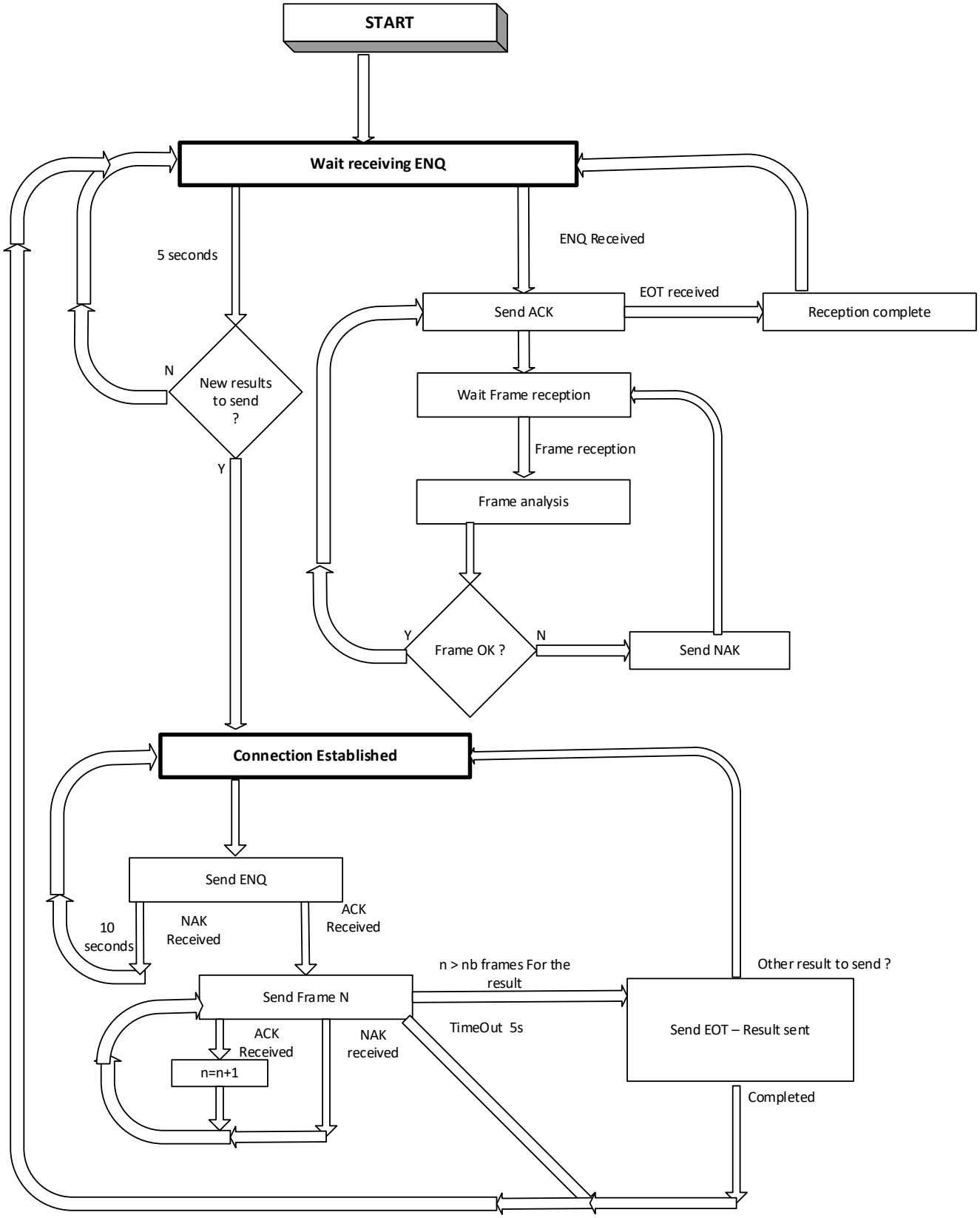
The purpose of this protocol is to send the Queries, (i.e. transform queries in frames exploitable by the LIS), to receive tests requests (i.e. transform frames received from the LIS in test requests), and finally return the results (i.e. transform results in frames exploitable by the LIS).

The frame format is:

<STX><Frame #><Data><ETX><Checksum><CR><LF>

(For more information about this protocol, visit www.astm.org)

The working schema is resumed in the next page.


START
Wait receiving ENQ
5 seconds
ENQ Received
EOT received
Send ACK
Reception complete
N
New results to send ?
Wait Frame reception
Frame reception
Y
Frame analysis
Y
Frame OK ?
N
Send NAK
Connection Established
Send ENQ
10 seconds
NAK Received
ACK Received
n > nb frames For the result
Other result to send ?
Send Frame N
TimeOut 5s
ACK Received
NAK received
n=n+1
Send EOT – Result sent
Completed

10.4.2. Logical protocol: ASTM E 1394

The Logical protocol ASTM E 1394 allows communication between LIS and Hb-Vario System:

- In Query mode, send the queries for sample identified onboard
- Receive Sample Ids and analysis to execute
- Send the results

(For more information about this protocol, visit www.astm.org)

10.4.2.1. Query

The query message is sent by the MAXMAT PL to request tests for a sample ID.
A query message is always made by:

- A « H » line (header)
- A « Q » line (query)
- A « L » (end of message1)

Example of query sent by the Hb-Vario for sample 05203002:

```
H|\^&|||HbVario|||||||P|E 1394-97|20150106142536
Q|1|05203002|||S|||||||O
L|1
```

Field table:

	Field	Content	Remarks/Value
H Line			
	Field n°1 :	Header ID	H
	2	Field delimiter	generally : |
		Repetition delimiter	generally : \
		Component delimiter	generally : ^
		Escape character	generally : & but unused
	3	Unused Field	
	4	Unused Field	
	5	Unused Field	
	6 => 11	Automate ID	MXT^1.1
	12	Processing ID	P (Patient) or Q(CQ)
	13	Version N°	E 1394-97
	14	Date & time of message	YYYYMMDDHHMMSS
Q Line			
	1	Q	
	2	sequence number	1
	3	Sample ID	
	4	unused	
	5	range	ALL
	6	Nature of request	S(specimen collect date), R(result

	Field	Content	Remarks/Value
			test date)
	7 => 12	Unused Fields	
	13	Request information status code	Generally: O
L Line			
	1	L	
	2	Sequence number	1

10.4.2.2. Test request

The test request message is sent by the LIS, either as an answer to a query when query mode is active, or when the sample and its analysis are recorded on the LIS.

It's mandatory that a test request message contains only analysis for only 1 patient.

If it's not the case, the request is analyzed and integrated, but an overload can result for the analyser (if, for example, the program tries to integrate 150 analyses for 20 patients during routine work …)

A test request message is composed by:
- H line (header)
- P line (patient)
- O line (order)
- L line (end of message)

Example:
```
H|\^&|||
P|1|00010032|||CLAUDE^DOMINIQUE|||
O|1|00010032||^^^HBA1C||||||||||
O|2|00010032||^^^VARIANT||||||||||
L|1
```

To configure codes and analysis matching, see paragraph 3.

Serial request:
When the request is made by several analysis, it's possible to use the field repeater symbol '\':

```
H|\^&|||
P|1|00010032|||CLAUDE^DOMINIQUE|||

O|1|00010032||^^^HBA1C\^^^VARIANT||||||||||
L|1
```

Pre-dilution:
If the sample tube is already a 'Lysate' sample, it is possible to inform the Hb-Vario:

```
O|1|00010032||^^^HBA1C||||||||||LYSATE
```

When an order contains the 'Lysate' information, the sample tube will be considered as Lysate and all analysis for this tube will be run in Lysate mode.

Action code:
Action code is used to cancel an analysis.

If a «C » is received in the 12th field of the O line, the analysis is canceled on the Hb-Vario.

This line:
`O|1|00010032||^^^HBA1C|||||||C||||`

Will cancel a creatinine request on the tube 00010032 (if this analysis is not already running or

10.4.2.2.1. Fields table:

	Field#	Detail	Value or remarks
H Line			
	Field n°1 :	Header ID	H
	2	Field delimiter	generally : |
		Repetition delimiter	generally : \
		Component delimiter	generally : ^
		Escape character	generally : & but unused
	3	Unused Field	
	4	Unused Field	
	5	Unused Field	
	6 => 11	Automate ID	MXT^1.1
	12	Processing ID	P (Patient) or Q(CQ)
	13	Version N°	E 1394-97
	14	Date & time of message	YYYYMMDDHHMMSS
Line P			
	1	P	
	2	Sequence number	
	3	Sample ID	
	4	Laboratory Patient ID	(optional)
	5	Patient ID	(optional)
	6	Patient Name	(optional)
	7	Unused Field	
	8	Patient birthdate (YYYYMMDD)	(optional)
	9	Sex (M, F, or U)	(optional)
Line O			
	1	O	
	2	Sequence number	
	3	Sample ID	
	4	unused	
	5	Analysis parameters :	

	Field#	Detail	Value or remarks
		component # 1,2,3 : unused	
		component # 4 : Analysis parameters	
	6 => 9	Unused Field	
	10	Tube Type	(optional)
	11	Unused Field	
	12	Code action	(optional) C to cancel a test
	13 => 15	Unused Fields	
	16	Specimen descriptor	LYSATE if the sample is already lysate
L Line			
	1	L	
	2	Sequence number	

10.4.3. Results

Once tests are completed, the Hb-Vario sends the results.

A result message contains only data for one sample, but a message may contain one or more result for one or more analysis.

A result message is composed of:
- H line (header)
- P line (patient)
- One O line or more (order)
- for every O line , one R line (result) or more
- M line (manufacturer)
- L line (end of message)

Example:

```
H|\^&|||HbVario|||||||P|E 1394-97|20150106142536
P|1|00010032|||CLAUDE^DOMINIQUE|||
O|1|00010032||^^^VARIANT|R||||Regular Primary||||||
R|1|^^^HBF|1.1|%||LOCONC|N|F||||20150106112502||

R|2|^^^HBA2|2.5|%||LOCONC|N|F||||20150106112933||
M|1|HBA1AB^223.2^2.1^-||
M|2|HBA1C^578.8^5.2^-||
M|3|HBF^115.5^-^1.1||
.
.
.
M|9||5780.2|
L|1
```

Error codes:

The 7th field of the R line contains a possible alarm code of the analysis.

Date/Time of execution: The 13th Field contains date and time when test has been completed on the Hb-Vario. This date format is YYYYMMDDHHMMSS

10.4.3.1. Fields Table:

	Field#	Detail	Value or remarks
H Line			
	Field n°1 :	Header ID	H
	2	Field delimiter	generally : |
		Repetition delimiter	generally : \
		Component delimiter	generally : ^
		Escape character	generally : & but unused
	3	Unused Field	
	4	Unused Field	
	5	Unused Field	
	6 => 11	Automate ID	MXT^1.1
	12	Processing ID	P (Patient) or Q(CQ)
	13	Version N°	E 1394-97
	14	Date & time of message	YYYYMMDDHHMMSS
Line P			
	1	P	
	2	Sequence number	
	3	Sample ID	
	4	Laboratory Patient ID	(optional)
	5	Patient ID	(optional)
	6	Patient Name	(optional)
	7	Unused Field	
	8	Patient birthdate (YYYYMMDD)	(optional)
	9	Sex (M, F, or U)	(optional)
Line O			
	1	O	
	2	Sequence number	
	3	Sample ID	
	4	unused	
	5	Analysis parameters:	
		component # 1,2,3 : unused	
		component # 4 : Analysis parameters	
	6 => 9	Unused Field	
	10	Tube Type	(optional)
	11	Unused Field	
	12	Code action	(optional) C to cancel a test
	13 => 15	Unused Fields	

	Field#	Detail	Value or remarks
	16	Specimen descriptor	LYSATE if the sample is already lysate
Line R			
	1	O	
	2	Sequence number	
	3	Analysis parameters :	
		component # 1,2,3 : unused	
		component # 4 : Analysis parameters	HBA1C, HBF, or HBA2
	4	result	• Numerical result • or '-' or '*' in non-reportable cases as reported in the application
	5	unit	
	6	Unused Filed	
	7	Error codes	Each error code is separated by ^
	8	Nature of abnormality	Generally: N
	9	Result status	Generally: F (Final)
	10 => 12	unused	
	13	Date/time completion	Format YYYYMMDDHHMMSS
M Line			
	1	Sequence number	
	2	Component #1: peak name	HBA1AB, HBA1C, HBF, HBA0…
		Component #2: peak area	• Numerical result
		Component #3: raw result	• Numerical result • or '-' or '*' in non-reportable cases as reported in the application
		Component #4: calibrated result	• Numerical result • or '-' or '*' in non-reportable cases as reported in the application
	3	Total area of the test	• Numerical result
	4	Chromatogram picture data	Each byte is separated by the component separator (^)
L Line			
	1	L	
	2	Sequence number	

10.4.3.2. Error codes Table:

The possible error codes that can be sent in the line R (field 7) are:

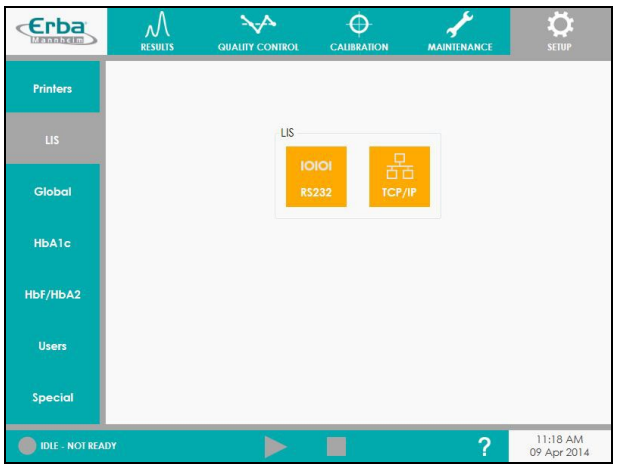
Flag	Detail	Result
OSAT	Optical saturation	'*'
CLOT	clot detected in low pressure system	Result + '*'
BADVOL	issue with blood column positioning into the loop	Result + '*'
2FAST	Sample transfer is too fast	Result + '*'
PCLOT	Sample transfer is too slow or partial clotting	Result + '*'
HICONC	High concentration (total area > 15000)	Result + '*'
LOCONC	Low concentration sample (total area < 2500)	Result + '*' Or only '-' if area < 1500
INCFLUC	incubator temperature fluctuation detected (not used for now)	Result + '*'
2OLD	Sample is too old, MetHb > 8.0 % RAW	Result + '*'
NOHBA0	No HbA0 detected (only in HbA1c mode)	'*'
NOHBA1C	No HbA1c detected (only in HbA1c mode)	'*'
HIHBF	HbF > 10.0 % raw (only in HbA1c mode)	Result + '*'
ABHBF	HbF > 2.2 % calibrated (only in HbA1c mode)	
ABHBA1C	HbA1c > 5.0 % raw (only in variant mode)	
ABHBA2	HbA2 > 5.0 % raw (only in HbA1c mode)	
HOMOZ	suspicion of homozygote	'*'
VARIANT	variant suspicion	Result + '*'
UNKNOWN	the sum of unknown peak > 3.0 % raw	Result + '*'

10.5.System Configuration

10.5.1. Configuration

In the « Setup »menu, the connection parameters are in the « LIS » tab.

Note: As soon as 1 of the 2 options is selected (black outlined), the LIS is considered activated, and the system will not program default analysis and replicate but await LIS requested tests.
To deactivate LIS connection, click on the selected button option to remove the black outline.



RS232:

To configure a serial communication (RS232) click on RS232 button:

 # Port: Specify in this field which COM port from the Hb-Vario instrument is used for the connection with the (Normally it is COM1).

Speed: Specify in this field the connection speed (Generally it is 9600 bauds).

Data bits: Specify in this field the data bits parameter (Generally it is 8).

Parity: Specify in this field the parity (Generally there are no parity, so select None).

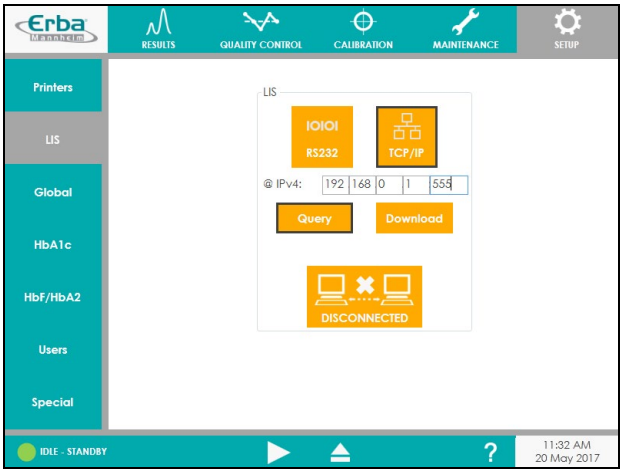
Stop bits: Specify in this field the number of stop bits (Generally it is One).

Flow: Specify in this field the flow control (Generally it is None).

Note: aside from **# Port** which is linked to the Hb-Vario instrument, all other pieces of information (speed, data bits, parity, etc.) are directly determined by the Host LIS system. The information in the Hb-Vario should match the Host LIS system.

Ethernet:

To configure an Ethernet communication (TCP-IP) click on TCP-IP button:



@IPv4: Specify in this field the host IP address and the socket number to establish the communication with the host.

For each kind of communication (RS232 or TCP-IP) you can choose to work in Query or Download mode.

- **Query:** The Hb-Vario will send a query to the host for every read barcodes.
- **Download:** The host will send all information once the connection is established. The Hb-Vario will never send queries, and it will run all tests where information is already in its database.

The LIS settings can be set or change at every moment but the connection is established automatically (if the RS232 or TCP-IP is selected) only when the system is initialized. If the connection needs to be activated manually after a Startup, click on this button:

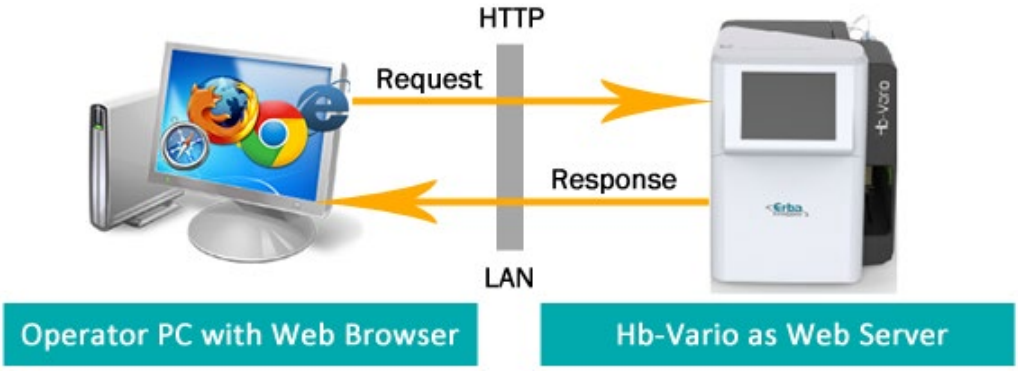
When the communication will be established, the button will change from:

To:

Once the communication with the host is established, it is possible to send manually results for a test from Result window or Quality Control Window.

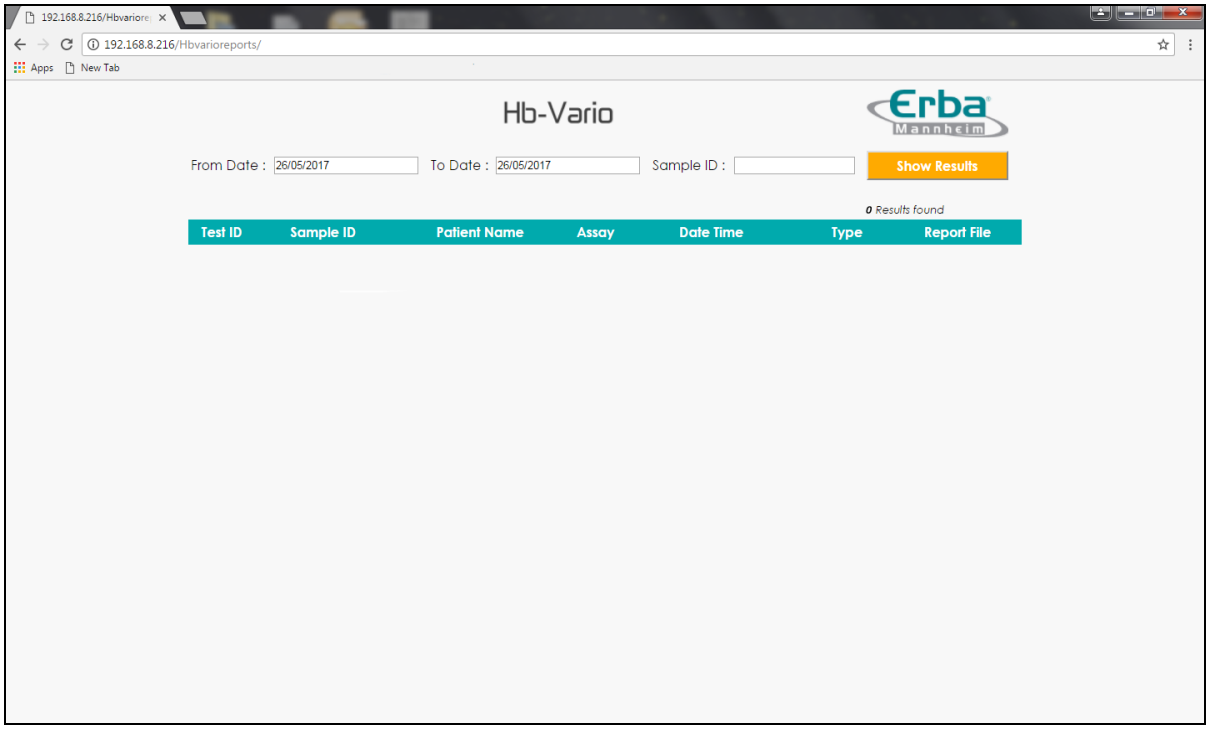
10.6.Web Utility

This option is used to transfer PDF reports containing the chromatogram and result table from the Hb-Vario instrument to the operator's computer.

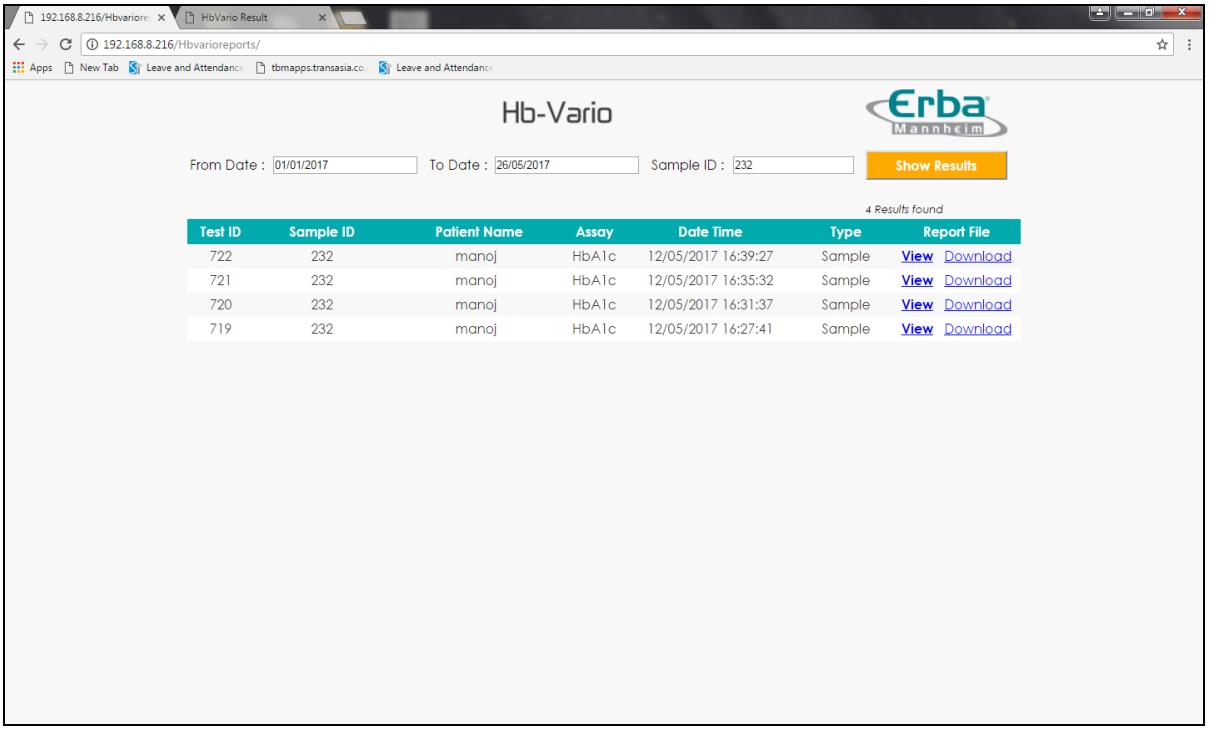


The Hb-Vario acts as a web server which hosts a single web page that can be accessed through a web browser on any PC connected on the same LAN as the Hb-Vario instrument.

URL of the web page will be formed using IP address of the Hb-Vario instrument (For example: http://192.168.8.216/Hbvarioreports) to gain access.

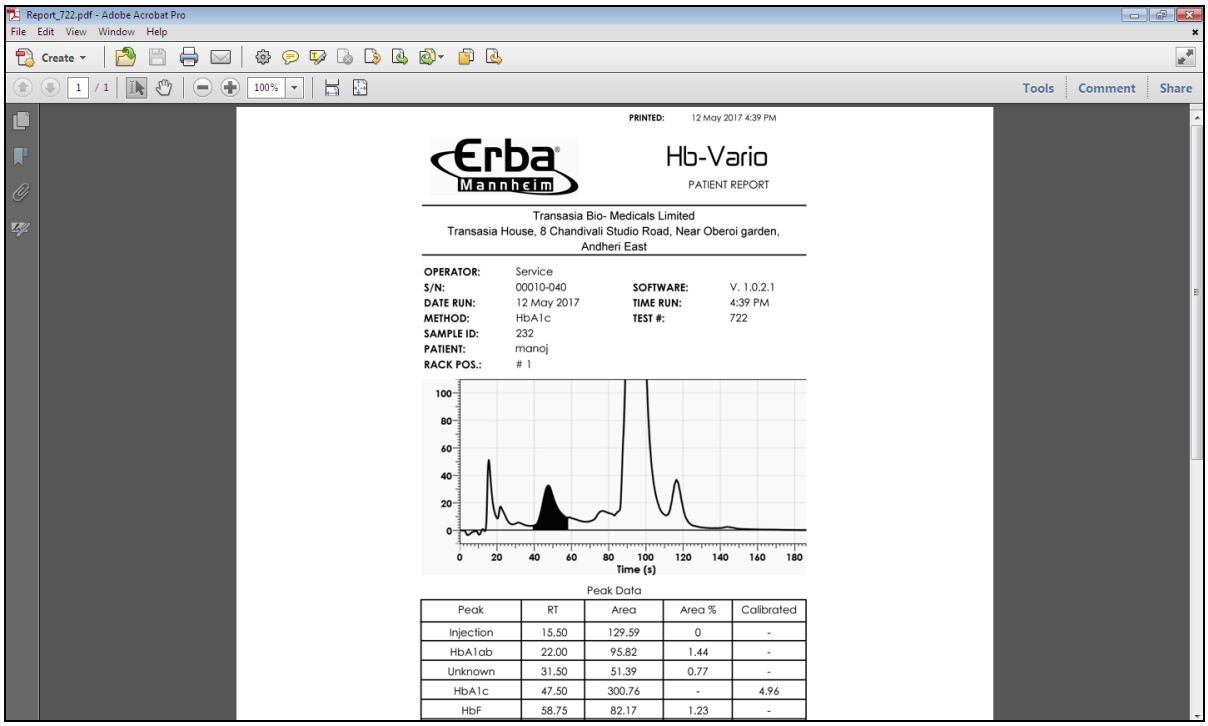


The web page has a result filter consisting of ***From Date: To Date:*** and ***Sample ID:*** for selection. Once filled, Click Show Results button. Results will be listed on the same page in a tabular form based on the corresponding filter selection.



Use View and Download links to view and/or download individual PDF reports of the corresponding Sample, Calibrator, Control, etc.

Clicking on View button the following window appears.

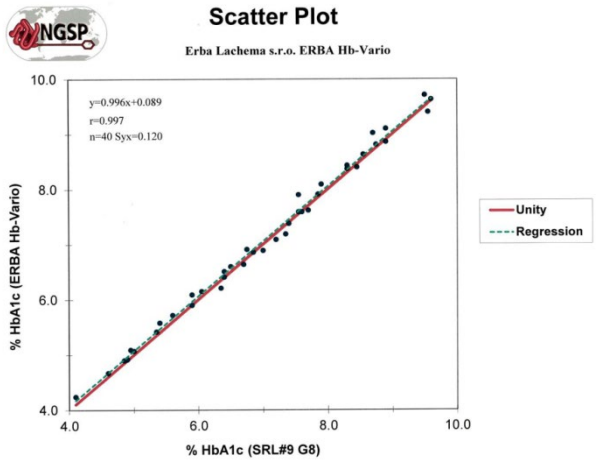


The PDF of the test report is displayed. Similarly, clicking on Download button saves the file to the Downloads folder of the Operator PC. Thus, enabling the user to view and share it on a later instance.

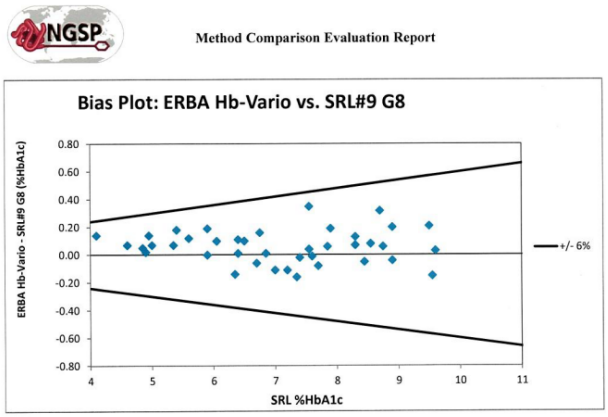
11 Performance

11.1. Accuracy and bias

The Accuracy and Bias as seen on the NGSP certification report exhibit a correlation with a R^2 of 0.997, a slope of 0.996 and intercept of 0.089 against the Tosoh G8.



Bias was within +/-6% on all 40 levels tested from a concentration of 4.1 to 9.6% as seen on the bias plot below.



The average bias on the 40 samples (range 4.1 to 9.6) was 0.059

The standard error of estimate of data was 0.12

The bias at the medical decision level was at or less than 0.2% of HbA1c (except of two samples).

11.2. Linearity and reportable range

The linearity was tested with 2 the extreme samples from a commercially available linearity kit at 3.8% HbA1c and 17.7% respectively.

Dilutions of the two samples together at different ratios result in a set of levels used to demonstrate the linearity of the Hb-Vario system across the reportable range. The resulted dilutions concentrations in HbA1c were calculated using their respective contributions corrected by their respective amount of total hemoglobin.

The Hb-Vario demonstrates to be linear from 3.8 to 17.7% with a R^2 of 0.9999 a slope of 0.9985 and intercept of 0.0538.

Level	Normal Low	Diabetic High	Theoretical value	Observed value	Percent recovery
1	100%	0%	**3.79**	3.79	100
2	80%	20%	6.35	6.45	99
3	60%	40%	9.02	9.08	99
4	40%	60%	11.79	11.79	100
5	20%	80%	14.68	14.75	100
6	0%	100%	**17.70**	17.70	100

Table 1: Linearity and recovery results

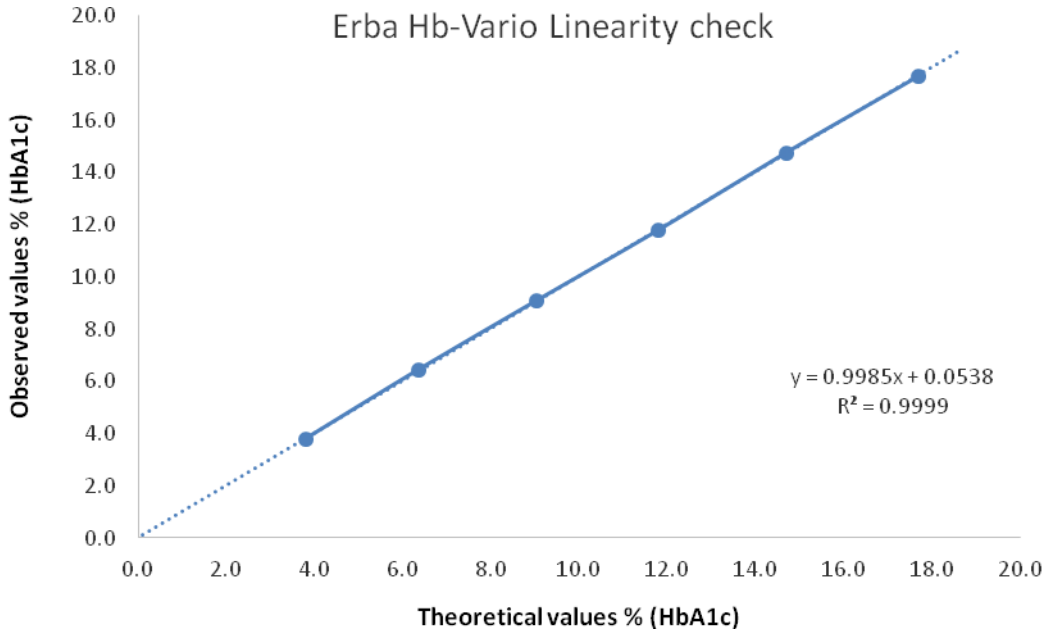


Table 2: Linearity and recovery graph

11.3. Analytical specificity (interferences)

11.3.1. Standard interferences

Several studies were performed to check the impact of specific potentially interfering substances on the % HbA1c results on normal and abnormal (>8% HbA1c) samples.
The 2 samples were either spiked with high dose of the interfering substance or increasing concentrations of substances. Unaltered blood sample (referred as controls in these studies) were systematically analyzed in parallel with the spiked samples (tests).
The acceptance criteria for interference substances was a variance in the %HbA1c value within the range of ± 6% of the original (free from interfering substance) value. The data are summarized below.

- Labile HbA1c – Free from interference tested up to 1500 mg/dL (10% of labile generated)
- Lipemia – Free from interference tested up to 1090 mg/dL
- Icterus – Free from interference tested up to 14.5 mg/dL
- Acetyl salicylic acid – positive bias above 40 mg/dL
- Carbamylated HbA1c – Positive bias at the lowest concentration tested of 20 mg/dL

11.3.1. Hemoglobin variant interferences

Hemoglobin variant interference studies were performed using patient samples containing HbAS, HbAD, HbAE and HbAC in comparison with results of the Trinity Primus.
The acceptance criteria for Hemoglobin variant was a variance in the %HbA1c value within the range of ± 10% of the result obtained with the method known to be free from interference on the variants. The data are summarized below:

- Abnormal F levels – No interference tested up to 10%
- HbAS – No interference up to 34.6% of S
- HbAC – No interference up to 36.5% of C
- HbAD – No interference up to 37.7% of D
- HbAE – No interference up to 26.3% of E

11.4. Carryover

The system demonstrates a carryover equal or less than 0.2% HbA1c.

A normal HbA1c patient sample with low total hemoglobin (7g/dL) was tested, and then tested immediately following an abnormal high HbA1c sample with high total hemoglobin. There was no variation in the results of either high or low HbA1c results.

To quantify the total carryover, blank samples were run immediately after patient samples. This study was conducted in whole blood and lysate, and crossed for all possible scenarios. The total areas were used to calculate the carryover. It resulted in less than 0.5% carryover in Whole blood and less than 0.9% carryover in lysate mode. The resulting impact is maximum 0.2% of HbA1c.

11.5. Whole blood versus lysate

For this study normal and abnormal (>8% HbA1c) were tested using the 2 modes Whole blood and Lysate. The results were compared for CV and variance.

The CV on the normal and abnormal samples were the same on the 2 modes, and the difference on the means were respectively 0.03% and 0.00% HbA1c.

11.6. HbA1c Precision

The **within run precision** consists of 20 replicates of 3 different samples (normal, decision point around 6%, abnormal >8% of HbA1c), the results are as follow:

	Normal	Decision point	Abnormal
Mean	5.4%	6.3%	8.3%
CV %	1.35	0.82	1.05

The **between-run precision** study consists of 3 samples (normal, decision point around 6%, abnormal >8% of HbA1c) run as whole blood for 10 working days followed by 10 working days running as whole blood. They were analyzed on 20 non-consecutive days.
They were run on one internal site on two instruments using two lots of reagents. It consisted in 2 analyses of each sample per run, 2 runs per day. The study lasted 29 days.
The results are as follow. The estimates of imprecision for each sample result obtained from all 3 study sites (all results averaged) analysis are given in the table below:

Actual number of days involved in the study, and number of sites:	29 days, 1 site
Actual total number of runs:	80 total runs
Number of observations:	240 observations
Number of instruments/devices used in the evaluation, and how results were pooled:	2 instruments, individual values not pooled
Number of reagent lots:	2 reagent/column lot numbers
Number of calibration cycles and calibration lots:	6 total calibrations once study started, 1 lot of Calibrator used.

	Normal	Threshold	Pathologic
Complete study (2 instruments, 2 lots)			
Mean HbA1c %	5.05	5.97	7.89
CV %	*3.12*	*2.76*	*2.2*
Instrument # 1, lot # 1			
Mean HbA1c %	5.05	6.04	7.88

CV %	*3.96*	*3.08*	*1.77*
Instrument # 1, lot # 2			
Mean HbA1c %	5.12	6.04	8
CV %	*2.82*	*2.67*	*2.4*
Instrument # 2, lot # 2			
Mean HbA1c %	5.02	5.88	7.83
CV %	*2.7*	*2.07*	*1.82*

11.7. Total hemoglobin concentrations

For this study the same 2 samples normal and abnormal (>8% HbA1c) were selected for their total hemoglobin value around 14g/dL. They were then altered by centrifugation followed by discarding part of the plasma and dilution its collected plasma to respectively create 3 levels of total hemoglobin for the 2 samples: 7 g/dL, 14 g/dL (unaltered), 21g/dL.

These samples were then analyzed in whole blood and lysate modes in parallel with control samples (unaltered) to check the variance and CV. The criteria are Variance +/-6% of HbA1c and CV <3%.

The study shows that on the normal and abnormal samples, there is no influence or significant difference in HbA1C results from 7.2g/dL to 20.2g/dL of Total Hemoglobin tested.

12 Disposal

12.1. End of life disposal

Before disposing the instrument, please contact the local Erba Lachema representative. Full instruction will be provided for instrument proper and complete disposal process in compliance of local and national regulations.

Note: A lithium battery is integrated on one internal electronic board.

13 Packaging

13.1. Transport requirements

Transport Environment limit ranges		
•	Temperature:	1-50°C
•	Humidity:	5- 90% (non-condensing)
•	Shock	< 35G

13.2. Packaging labels

The labels applied on the external packaging specify the environmental conditions acceptable for transportation and storage of the device, as described in this manual.

They also contain necessary labels for the package to be handled appropriately during transports like FRAGILE, this side up, etc. as needed, according to internal procedures.