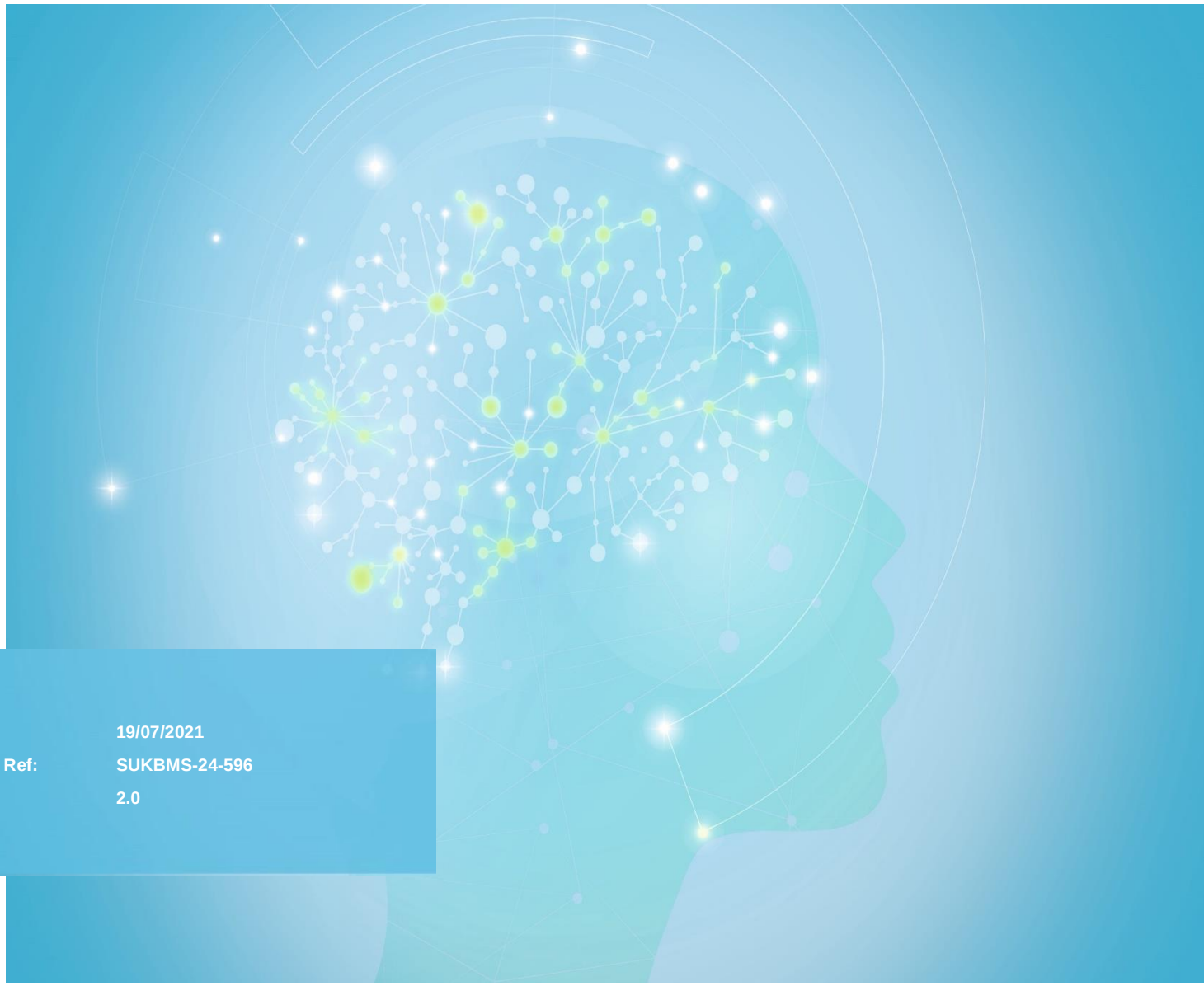
Routine Use Training Workbook

TOSOH G8



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Sysmex House, Garamonde Drive, Wymbush, Milton Keynes, MK8 8DF, United Kingdom · Phone +44 333 320 3460 · Fax +44 1908 267 901
academy.training@sysmex.co.uk· **www.sysmex.co.uk**

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Disclaimer

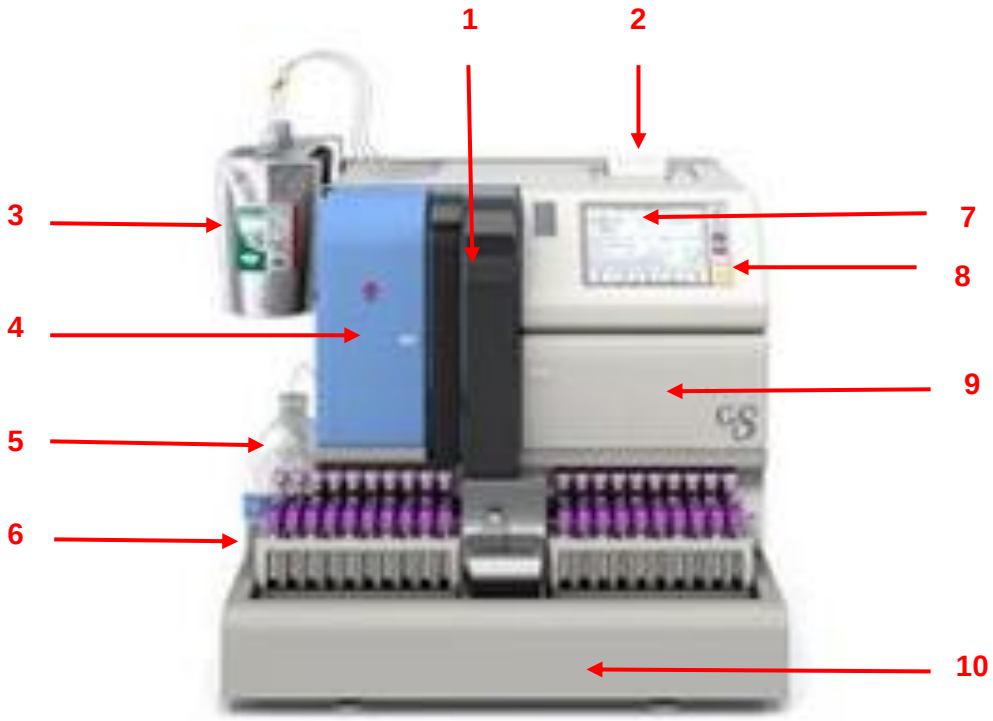
Please note, the information contained in training resources provided by Sysmex should not be used as an alternative to your sites Standard Operating Procedure (SOP)/Contract. If you have any particular questions regarding any site specific use of reagents, consumables and/or equipment please contact your Management Team.

TOSOH G8 Overview

The TOSOH G8 is a full automated HPLC analyzer that can be used to perform HbA1C or haemoglobinopathy analysis (standalone only). The analyzer can fully integrated with Sysmex XN9000 system.

Facts and Figures	
Sample type	EDTA
Minimum sample volume	1ml
Aspiration volume	4µl
Technology	High performance liquid chromatography (HPLC)
Modes	System mode Sampler mode Pre-dilution mode
Parameters	HbA1a HbA1b HbF I-A1c S-A1c A0
Throughput **XN track**	 30 samples per hour
Analysis time	3.5mins for first result from standby 1.6mins per sample thereafter
Linearity **G8 Variant mode (SA1c area)**	 700-3000 (Recommended) 500-4000 (Acceptable)

Analyser Components



1. **Sampling unit** – houses the needle unit which is responsible for cap piercing and aspiration of blood samples. The cover to the sampling unit should NOT be removed while the analyzer is powered ON.

2. **Thermal printer** – used for printing out chromatograms, error messages and parameter status.

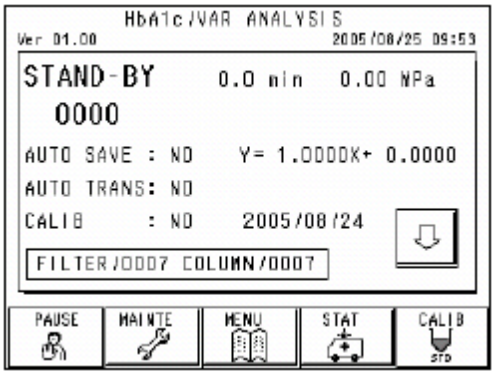
3. **Buffer reagents** – There are 3 buffer reagents housed on-board the analyzer, which work in combination to elute HbA1a, HbA1b, HbF, SA1c, LA1c and any other haemoglobin variants present.

4. **Drain valve housing** – the drain valve (C) is located under the cover and is responsible for removal of air from the analyzer, when required. The drain valve should be closed during analysis (as pictured). Also located under the cover are the Rotary valve (A) and the Injection valve (B).

5. **Haemolysis wash** – used for the breakdown of red cells and in manual dilution of samples.

6. **Mains power switch** – used to turn the mains power ON and OFF.

7. **LCD screen** – user interface for operation of the TOSOH G8. Contains the analyzer settings, reagent/consumable information, and version information.



8. **Control Panel**

Power Switch – use to power down and start up the analyser

START – Starts analysis

STOP – Stops analysis

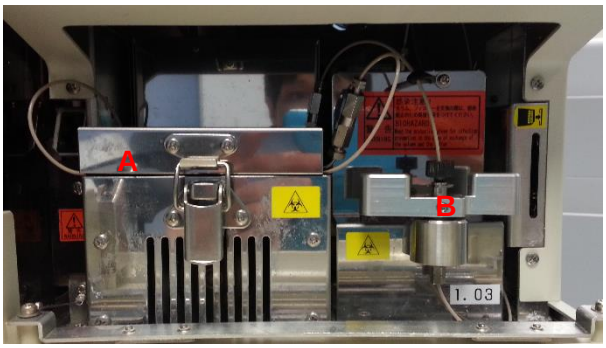
HOME – Returns LCD screen to main screen

E.RESET – Silences alarm

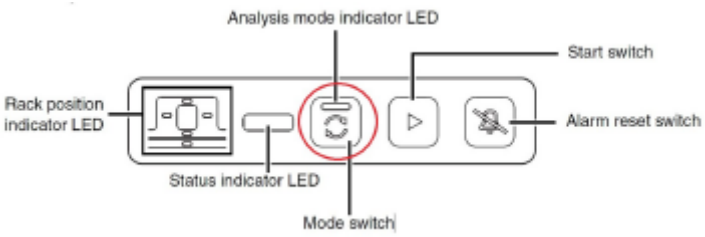
9. **Column and filter housing**

A. Column oven – the column oven contains the column, which must be kept at a constant temperature (25ºC) for accurate analysis.

B. Line filter – protects the analyser by preventing impurities from entering the assay line. The filter must be replaced approx every 400 cycles.

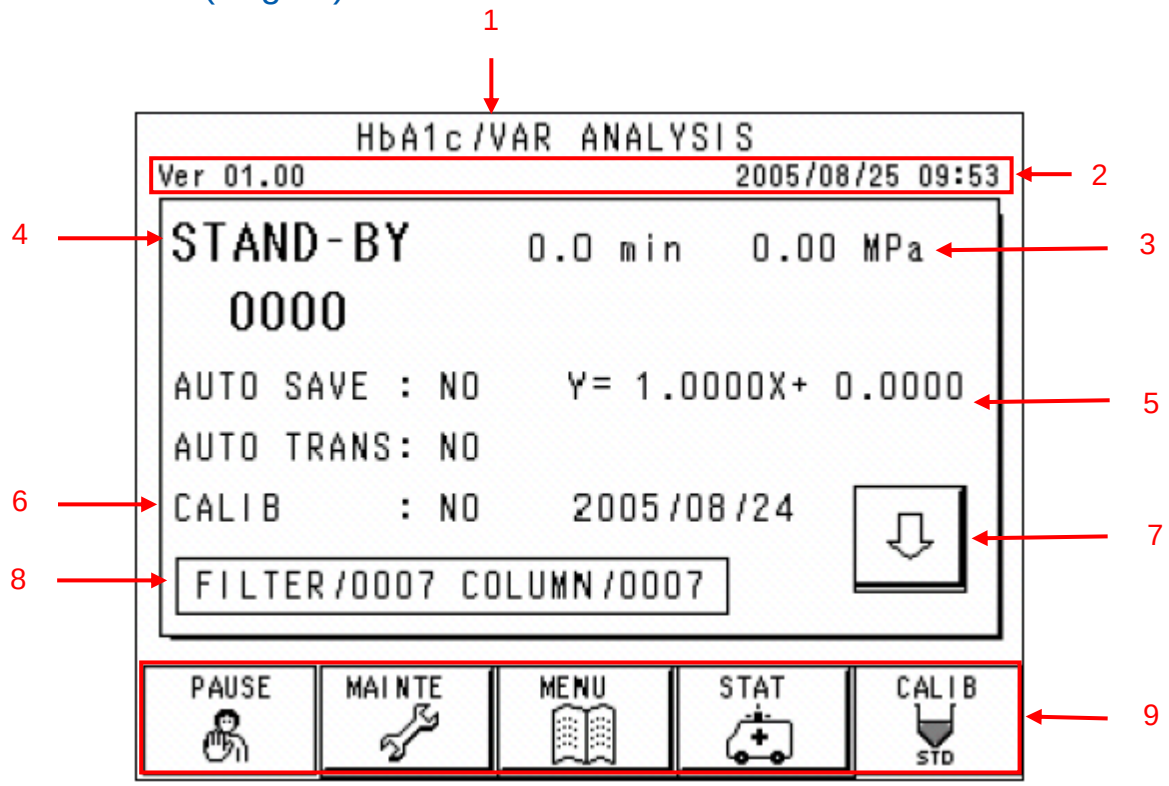


10. **XN Track/Sampler unit** – Samples are placed in racks and are introduced from the XN-9000 track when the analyzer is in 'system mode'. If required the analyzer can be placed into 'offline mode' using the [Mode Switch] on the control panel of the conveyer unit (CV-60). A GREEN mode switch indicator LED indicates 'system mode' and an ORANGE LED indicates 'offline mode'.



LCD Screen Layout

Main Screen (Page 1)



1. **Mode** - displays the assay mode the analyzer is currently in;

   [HbA1c/VAR ANALYSIS]: used for HbA1c analysis
   [B-THALA ANALYSIS]: used for haemoglobinopathy analysis

2. **Version number, date and time**

3. **Pump flow pressure** – The pressure should remain initial pressure ±4. The initial pressure target can be found in the insert included in the column box.

4. **Analyzer status** - displays analyzer status;

   [WARMING UP]: Displayed when analyzer is starting up.
   [STAND-BY]: The analyzer will enter STAND-BY when WARMING UP or WASH operation are complete. During STAND-BY the pump is stopped to prevent elution buffer being used. If the analyzer is not used for a maximum of 3hrs, the analyzer will automatically turn 'OFF'.
   [ANALYSIS]: Displayed during analysis of patient samples, QC or calibration. Following ANALYSIS the analyzer will go into WASH status.
   [WASH]: Displayed when analysis is complete. The WASH process takes 3 mins and involves pumping of buffer No.3 for 1min and No.1 for 2mins. On completion the analyzer will go into STAND-BY.

[BUFF PRIME]: Displayed following power ‘ON’ or following reagent changing. During BUFF PRIME 5ml of each elution is primed into the flow line.
[PUMP CLEAN]: Displayed following WASH. Plunger seal is automatically washed with haemolysis wash solution to prevent contamination or buildup of salt precipitate.

5. **Calibration factor** - Calculated during calibration analysis.

6. **Calibration Status;**

   [NO]: Calibration not being performed. Last calibration date.
   [YES]: Calibration being performed

7. **Next** – used to navigate to additional screen.

8. **Filter and column counts**

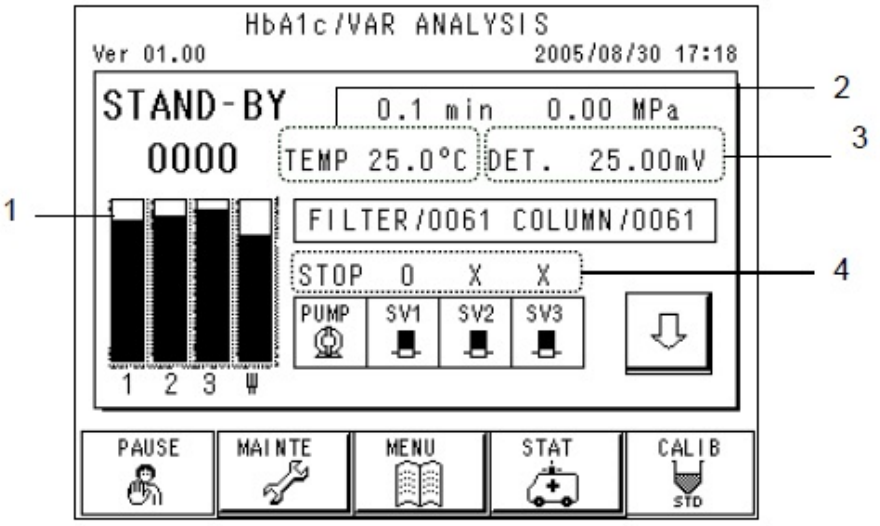
   [Filter]: number of injections since filter was replaced. The filter should be replaced approximately every 400 injections (See page 16)
   [Column]: number of injections since column was last replaced. The column is guaranteed for 3000 injections (See page 17)

9. **Menu options** – used to navigate through various menu options

   [PAUSE]: not for use on tracked analyzers
   [[MAINTE]: used to register reagent changes
   [MENU]: used for creating barcode IDs and placing analyzer into ‘Pre-diluted’ mode
   [STAT] Not available on tracked analyzers
   [CALIB]: used for checking and setting calibration values

Main Screen (Page 2)

Main screen (page 2) can be accessed by pressing the next button [↓] on main screen (Page 1)



1. **Reagents** – graphical display of remaining reagent volumes.
2. **Column oven temperature** – displays current column oven temperature.
3. **Detector output** – displays detector output.
4. **Operation status of the pumps and solenoid valves**

PUMP : Starts or stops pump run (STOP: stop pump FLOW: run pump)

SV1 : Opens or closes the valve for Elution Buffer No. 1
(o: opened x: closed GE: Gradient elution)

SV2 : Opens or closes the valve for Elution Buffer No. 2
(o: opened x: closed GE: Gradient elution)

SV3 : Opens or closes the valve for Elution Buffer No. 3
(o: opened x: closed GE: Gradient elution)

Reagents On-board the TOSOH G8

The TOSOH G8 has 4 on-board reagents, 3 elution buffers and 1 haemolysis wash, which are used during analysis. The 3 elution buffers are used in combination during analysis for separation of the haemoglobin variants and are in colour code bags. The Lot letter of the elution bags should always match the lot letter of the column in use.

Reagent	Function	pH	Salt conc.	Colour
Buffer 1	Low ionic strength buffer. Elutes off weakly bound fractions like HbA1a and HbA1b	Low	Low	GREEN
Buffer 2	Elutes off HbF, I-A1c and s-A1c.	Low	Medium	RED
Buffer 3	Elutes off all remaining fractions	High	High	YELLOW
Haemolysis wash	Responsible for the haemolysing red cells and the release of haemoglobin. Also used in on board dilation of whole blood samples (1:200)	N/A	N/A	N/A

***IMPORTANT**: The lot letter of reagents are available for approximately 2 yrs. The lot letter of the reagent bag should always match the lot letter of the column.*

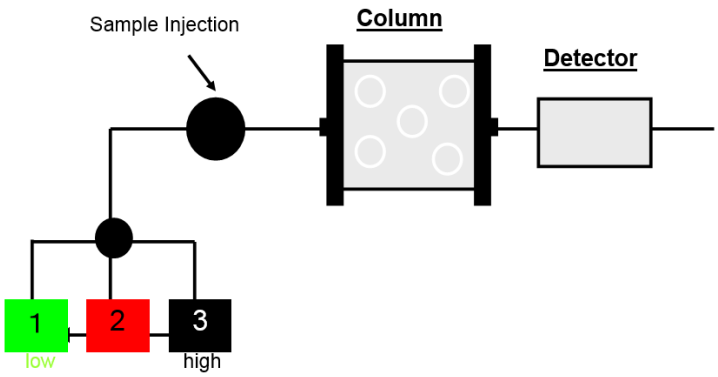
***NOTE**: The background of lot letter for Hb variant mode are solid colours. Bags with strip backgrounds to the lot letter are the buffers for Beta Thal mode.*

Analysis Principles and Parameter Production

High Pressure Liquid Chromatography

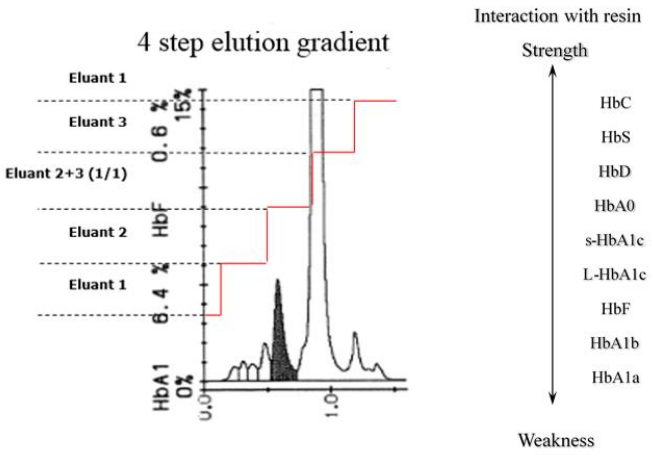
The TOSOH G8 uses high pressure liquid chromatography (HPLC) in order to separate the haemoglobins found within whole blood. It does this through a combination of liquid and stationary phase chromatography. The stationary phase is provided by the column, which is coated with ion exchange resins coated with charged groups, making it acidic in nature. The liquid phase is provided by stepwise elution of the eluents, which provide a linear gradient of pH and salt concentrations.

Following aspiration of 4µl whole blood the specimen is diluted with haemolysis wash 1:200 ratio. Haemolysis wash is then responsible for the haemolysis of the red blood cells and the release of the haemoglobin for analysis. The diluted sample is injected into the column. The positive charges on the haemoglobins interact with the negative charges of the resins inside the column.



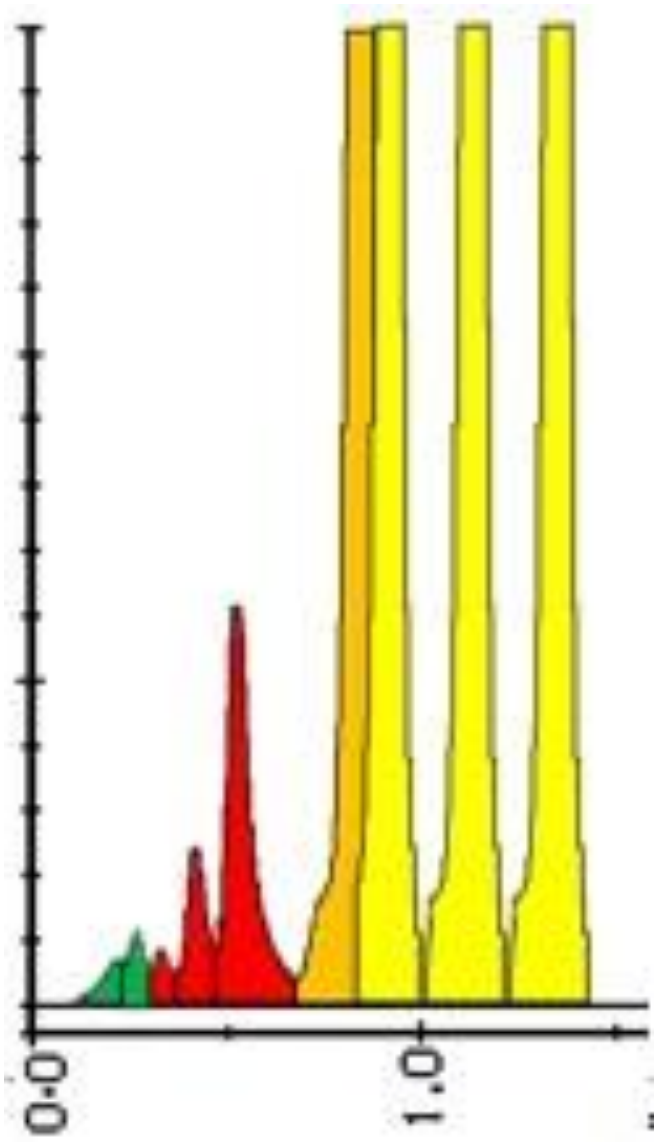
The addition of eluents in a specific order results in the elution of haemoglobin fractions with the weakest bound fractions eluting first.

Buffer 1 has the weakest ionic strength and is added first to the column resulting in the elution of weakly bound fractions such as HbA1a and HbA1b. Buffer 2 is then added which has a stronger ionic strength than Buffer 1 and is responsible for the elution of HbF, I-A1c and s-A1c. Buffer 2 and 3 are then added in 1:1 ration allowing the creation of a concentration gradient due to the high ionic strength of buffer 3. This results in the elution of HbA0. The final step is the addition of Buffer 3 only, causing the elution of any remaining strongly bound haemoglobin fractions. Each haemoglobin fraction then passes in turn through the detector where their respective absorptions are read by bichromatic detector. The samples are read at 415nm and a reference reading is taken at 500nm.



Task 1: Analysis Principles and Parameter Production

Using the information provided annotate the chromatogram below, labeling the axis, peaks and identifying reagents used:



Maintenance

Daily Checks

Before using the analyzer a series of checks should be made;

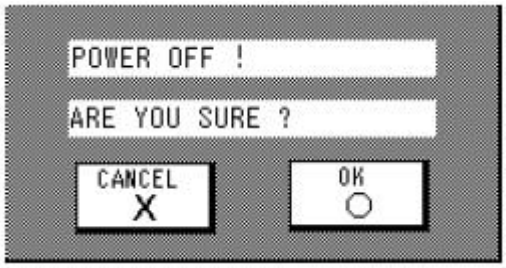
- Check reagent levels
- Check filter and column injection counts
- Check pressure
- Waste (if required)

Pump Clean (Daily Shutdown)

The analyzer should be turn OFF on a daily basis in order to perform a pump clean. The pump clean is performed as part of the background checks following power up.

In order to shut down the analyzer, ensure that no work is being processed and the analyzer is in STANDBY. Place the analyzer in to ‘sampler mode’ using the mode switch on the conveyor unit (See page 8).

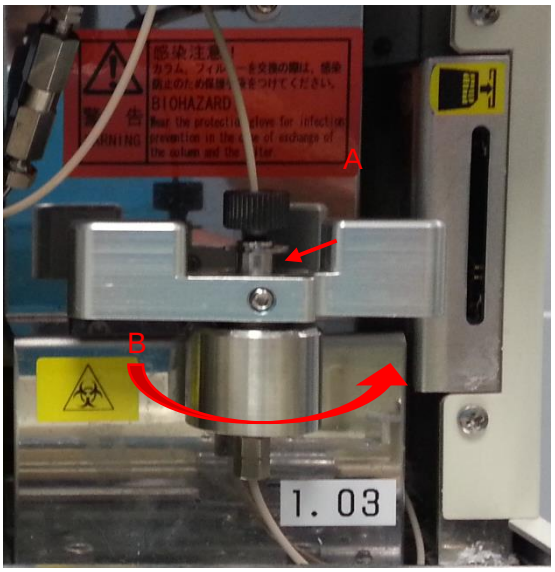
To shut down the analyzer press the [POWER] switch on the front of the analyzer. The following message will appear:



Press [OK]. Once analyzer is fully powered down press the [POWER] switch to start the analyzer back up. On start up the analyzer will perform a series of background checks including a pump clean, priming of the buffers and washing of the aspiration line. This is represented by the analyzer status changing from PUMP CLEAN to BUFF PRIME to WARMING UP. Completion of the startup procedure takes approx. 9mins. Once WARMING UP is complete the analyzer will go into STANDBY mode and is then ready for use. Place analyzer back in ‘system mode’ (See page 8).

Replacing the Line Filter

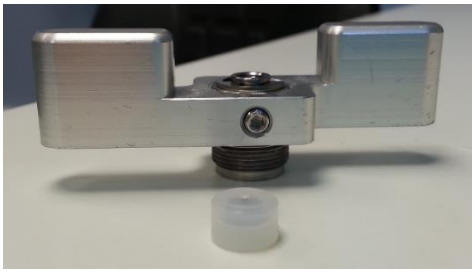
The line filter should be replaced every 400 injection or when the pressure is ±4 from the stated target (See column insert). ***IMPORTANT****: The analyzer will alarm at 400 injections and will continue to alarm with every injection over 400. Failure to replace line filter when appropriate may result in damage to the column and analysis errors.*

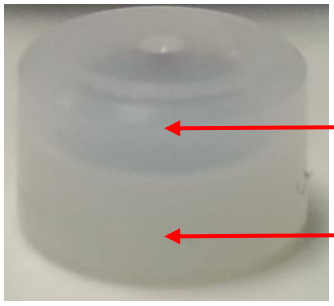


In order to replace the line filter the analyzer needs to be placed into Sampler mode (See page 25) and in STANDBY. Open the 'column and filter housing' by pressing on the cover. Unscrew the black filter outlet (A) and remove the filter holder (B) by turning the filter holder counterclockwise.

Once removed from the analyzer push down on the filter release to remove filter. Once filter has been removed discard the filter.

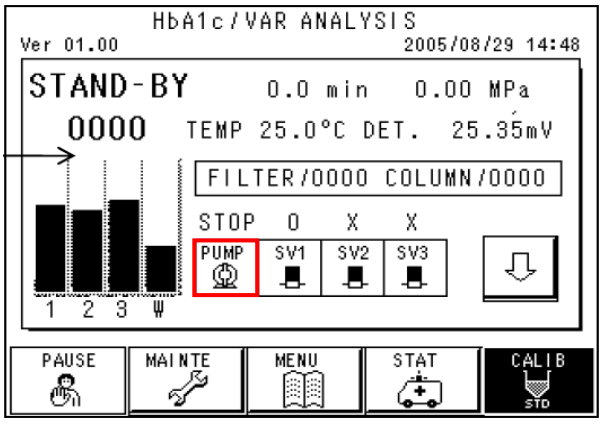
To replace the filter, place a new filter on a flat surface with the white side facing down.





Place holder on top of filter and push down. ***NOTE****: if the filter is orientated in the correct direction it will easily go back into position and the filter will sit proud within the holder. If there is resistance to the filter, check the filter is orientated in the right position (See picture above)*. Screw the filter holder back in place and re fit the filter outlet. Before using the TOSOH G8 perform a pump prime to check for leaks and ensure pressure is in the correct range.

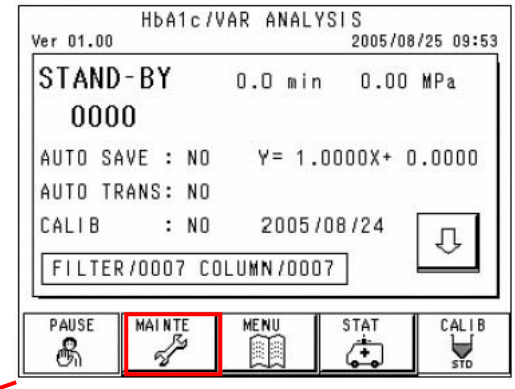
A pump prime can be performed from the second screen of the main menu by pressing [PUMP]. To stop the pump press [PUMP] again. Close column and filter housing cover.

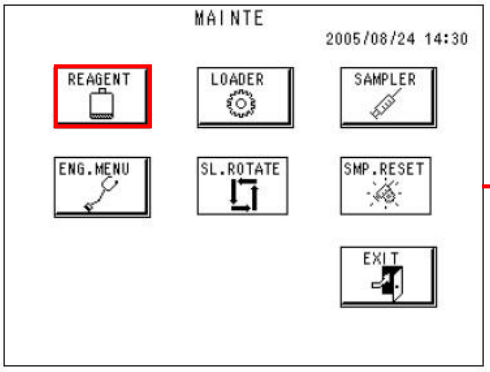


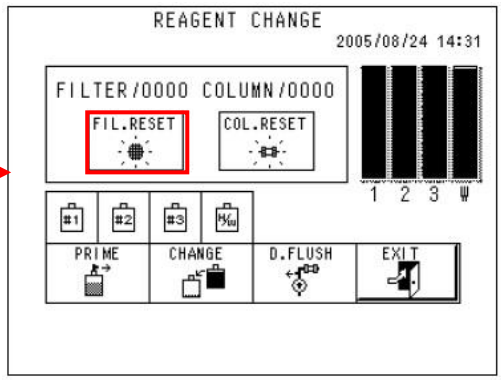
To reset the filter count to zero from the main screen select [MAINTE] then [REAGENT].

This will open the 'REAGENT CHANGE' screen, press [FIL.RESET].

Perform QC to ensure analyzer is working correctly (See page 21).



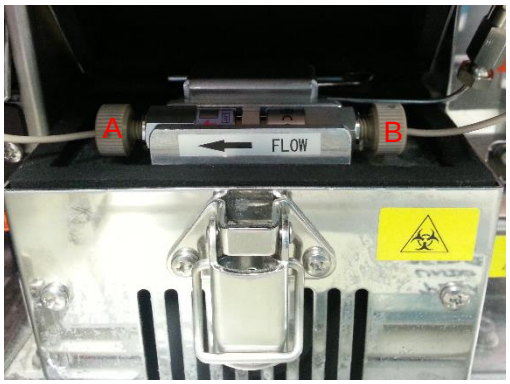




Changing the Column

Columns are guaranteed for 3000 injections, however they can be run till between 5000-8000 injections as long as QC and chromatograms remain okay. Each column has a unique serial number and the lot letter of the column should match the lot letter of the reagent being used. The column is located within the column oven, which ensures that the temperature is kept at 25ºC. ***NOTE****: large fluctuations in temperature have an effect on the production of results.* Following column replacement the column count MUST be reset to zero and column calibration MUST be performed.

To change the column ensure the analyzer is in 'sampler mode' and in STANDBY. Open the column and filter housing and unscrew the inlets from detector side (A) and the pump side (B) and remove column from the column oven. Discard old column according to laboratory protocol.

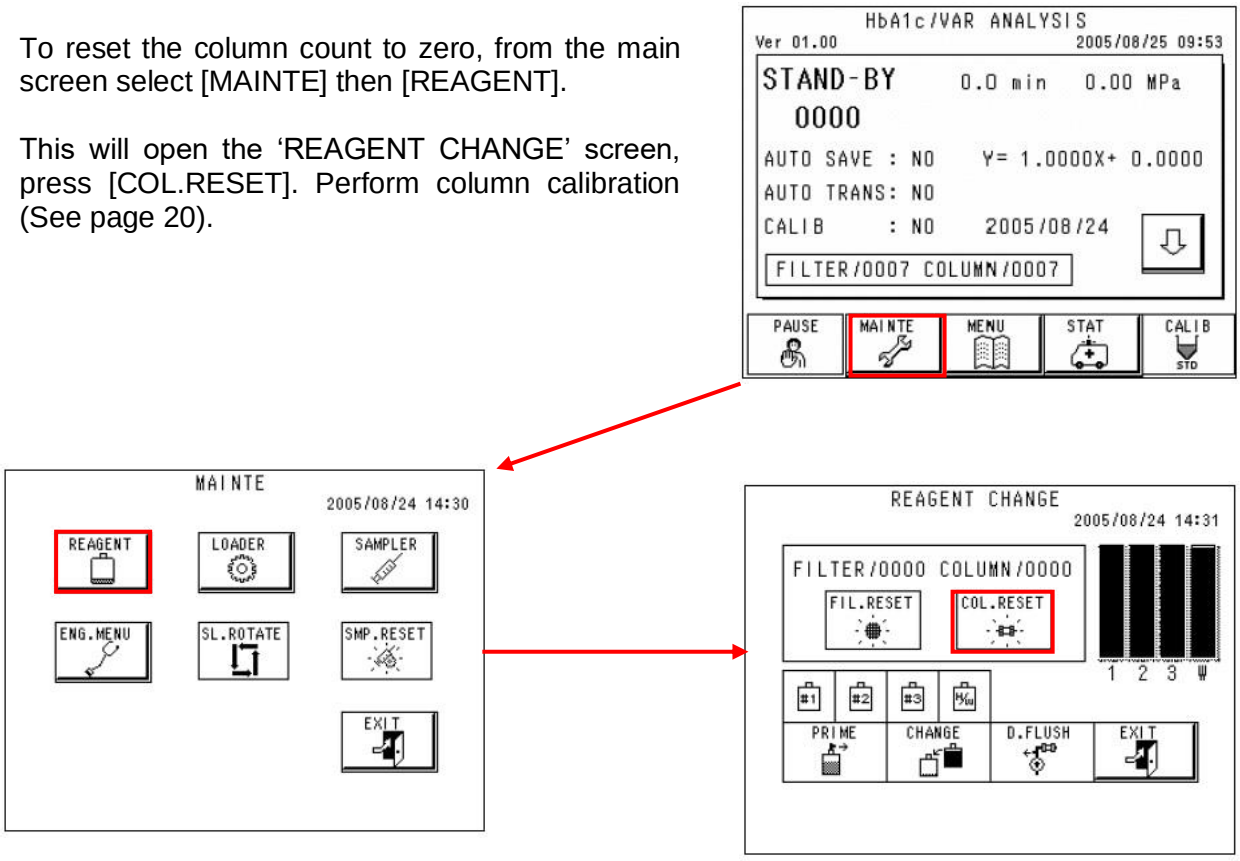


Remove protective plugs from the new column and reattach detector and pump inlets ensuring the arrow on the column is placed in the direction of the flow, e.g. from pump side (B) to detector side (A).

Before using the TOSOH G8 perform a pump prime to check for leaks and ensure pressure is in the correct range. A pump prime can be performed from the second screen of the main menu by pressing [PUMP]. To stop the pump press [PUMP] again. Close column oven and column and filter housing cover.

To reset the column count to zero, from the main screen select [MAINTE] then [REAGENT].

This will open the 'REAGENT CHANGE' screen, press [COL.RESET]. Perform column calibration (See page 20).



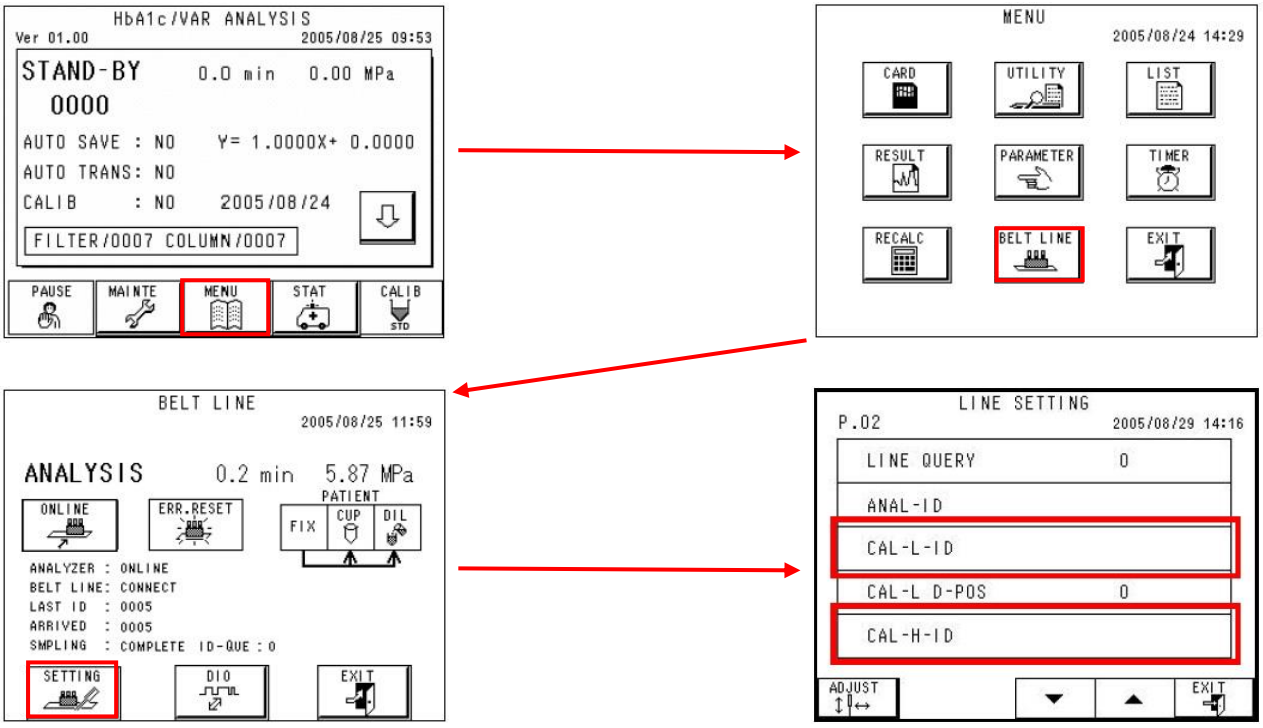
Column Calibration

Column calibration should be performed following

- Column replacement
- When there is evidence of QC drift
- Following major analyzer maintenance

Registering a New Lot of Calibration Material

Before a new lot of calibration material can be used on the TOSOH G8 a new 13 digit barcode must be created. This can be done by entering the [MENU] screen from the main menu, followed by pressing [BELT LINE] then [SETTINGS].



Select each calibrant in turn, ‘CAL-L-ID’ and ‘CAL-H ID’ for the low and high calibrators respectively, and enter the desired 13 digit barcode, for example Lot No. ACAL01 enter 7 spaces followed by ACAL01 (_,_,_,_,_,_._ACAL01)

Preparation of Calibrator Material

During calibration two levels of calibrator are used;

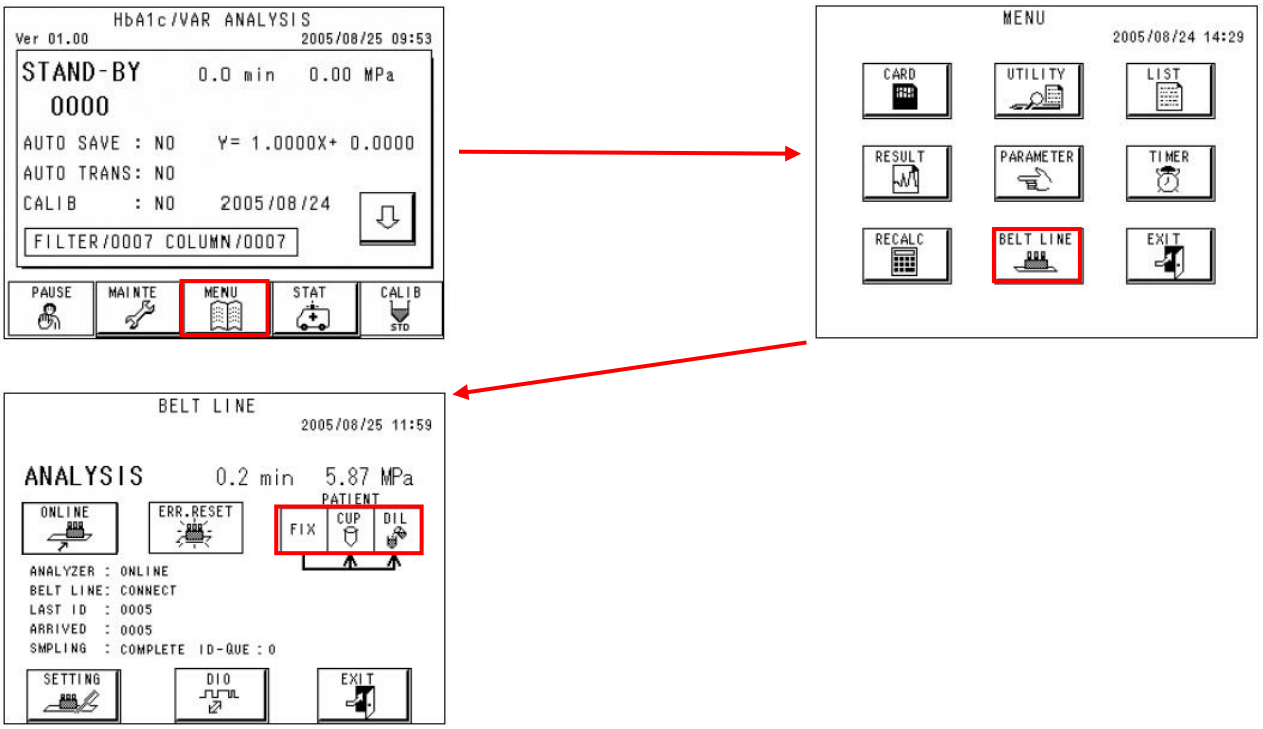
- Level 1 = Low HbA1c
- Level 2 = High HbA1c

The material is lypholized human haemoglobin and one vial should be made up using 4ml of distilled water. Once reconstituted the material can be aliquoted in to 1ml aliquots and frozen for a

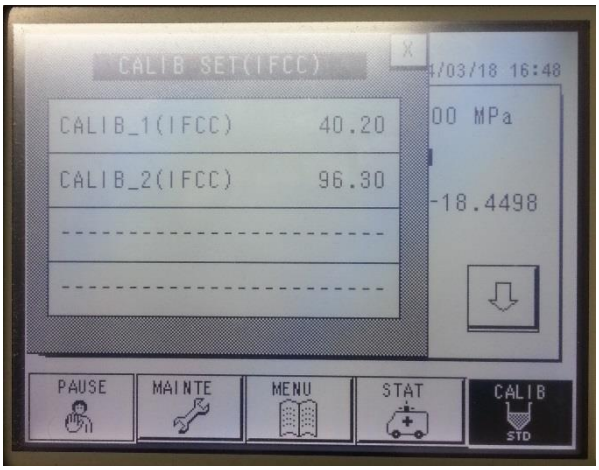
maximum of 30 days. ***NOTE**: if kept in resealed vial in fridge calibrator is stable for 7days. Do **NOT** leave calibrator at room temperature for long periods of time.*

Performing Calibration

In order for column calibration to be performed, ensure the analyzer is in STANDBY, the analyzer is in 'sampler mode' and the [BELTLINE] the FIX, CUP and DIL ***DO NOT*** have to be highlighted.



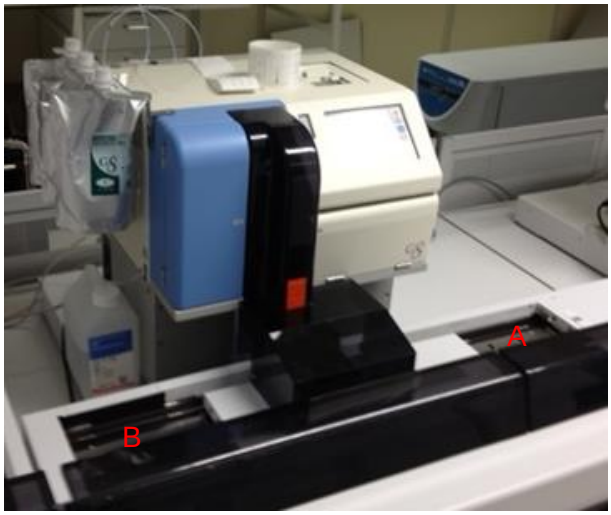
Once isolated check the calibrator values in the G8. Calibration values can be found on the calibration kit insert. From the main menu screen press [CALIB] to enter the 'CALIB SET' Screen and check assigned values. If values are incorrect, select the appropriate calibrator and edit value using the keyboard displayed, pressing [ENTER] to confirm.



Place Level 1 calibrator in an appropriately barcoded cup in position 1 of the rack and calibrator 2 in position to of the rack in an appropriately barcoded cup.

Place the rack on the input area on the right side of the conveyor (A) and press [START] button on TOSOH G8. Once aspiration of calibrators is complete the rack will move to the output area on the left side of the conveyor (B).

During analysis, the level 1 calibrator is analyzed 3 times (runs 1 to 3) and level 2 calibrator is analyzed twice (runs 4 to 5). The first analysis of calibrator 1 is discarded and the calibration factor is calculated from the mean %sA1c of analysis 2 and 3 for level 1 and analysis 4 and 5 for level 2. These values are then compared to those with the TOSOH G8 settings.



On completion of calibration analysis the new calibration factors will appear on the main screen and calibration status will display 'COMPLETE' and the date calibration occurred. Calibration data will be printed out. ***TIP****: A good calibration is indicted by the first figure being 12.5 and below and the second figure being between -16 to -18.*

Once analysis is complete, place the analyzer back into 'whole blood' mode (See page 25) and back into 'system mode' (See page 24). ***NOTE****: if analyser left with FIX, CUP and DIL highlighted whole blood samples will not be diluted during analysis this will lead to AREA TOO HIGH and possible damage to the column.*

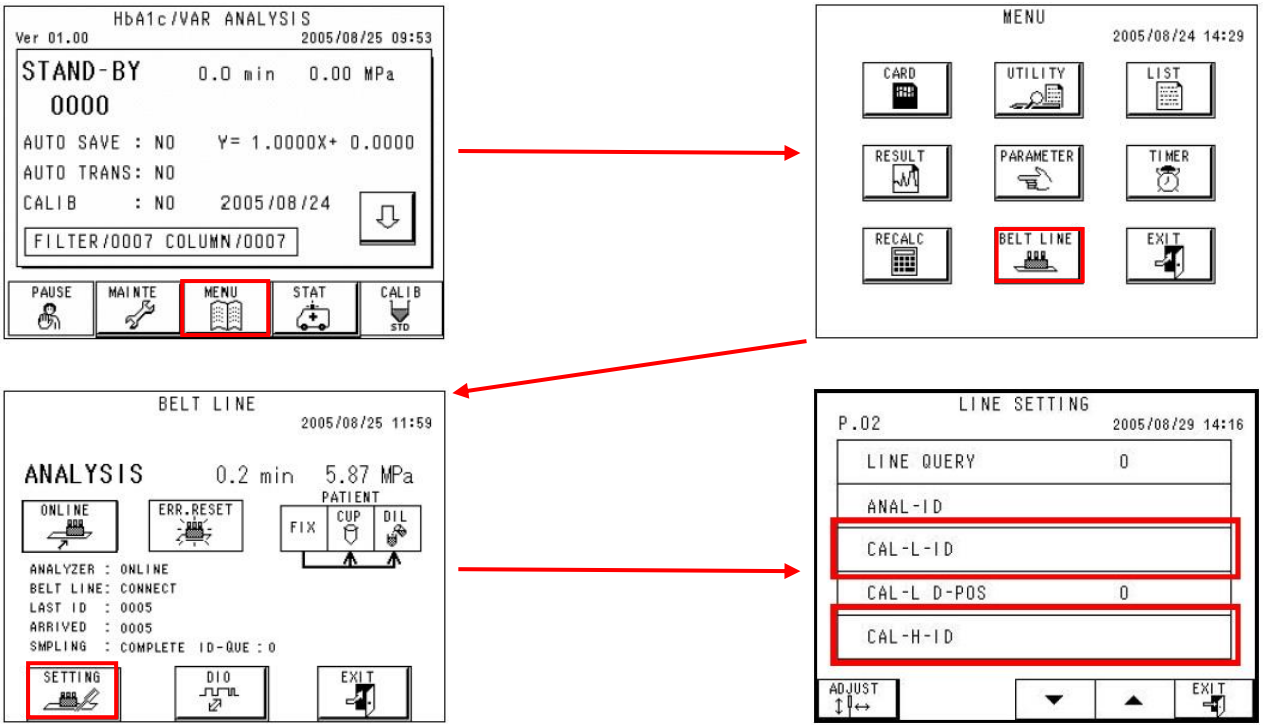
Quality Control (QC)

Quality control (QC) should be performed following calibration of the analyzer, troubleshooting or as part of daily checks/maintenance.

The material is lyophilised material and should be made up according to manufacturer's instructions.

Registering a New Lot of QC

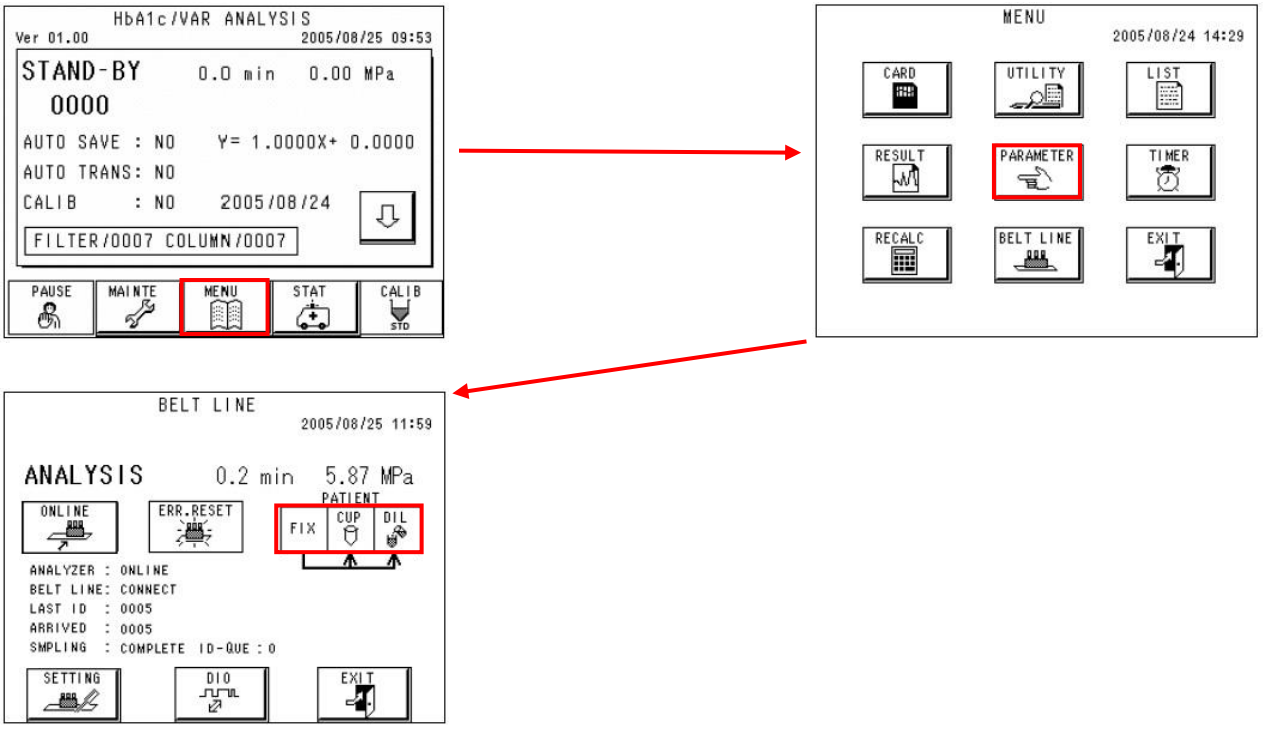
Before a new lot of QC can be run on the TOSOH G8 a new 13 digit barcode must be created. This can be done by entering the [MENU] screen from the main menu, followed by pressing [BELT LINE] then [SETTINGS]



Select each QC in turn, 'QC-L1-ID' and 'QC-H1-ID' for the low and high QC respectively, and enter the desired 13 digit barcode, for example Lot No. AQC01 enter 8 spaces followed by AQC01 (_,_,_,_,_,_._,_AQC01)

Running QC

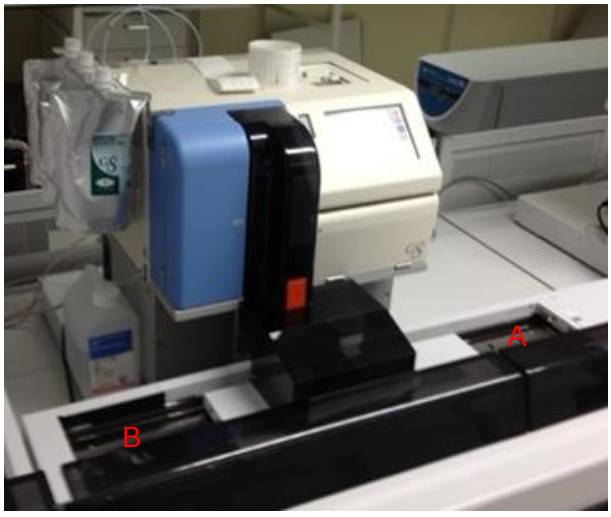
In order to perform QC, ensure the analyzer is in STANDBY, the analyzer is in 'sampler mode' and the [BELTLINE] the FIX, CUP and DIL have been highlighted.



Place 1ml of distilled water and 20µl of QC material in appropriately barcoded cups and mix thoroughly before placing in the rack.

Place the rack on the input area on the right side of the conveyor (A) and press [START] button on TOSOH G8. Once aspiration is complete the rack will move to the output area on the left side of the conveyor (B).

Once analysis is complete place the analyzer back into 'whole blood' mode (See page 25) and back into 'system mode' (See page 24).



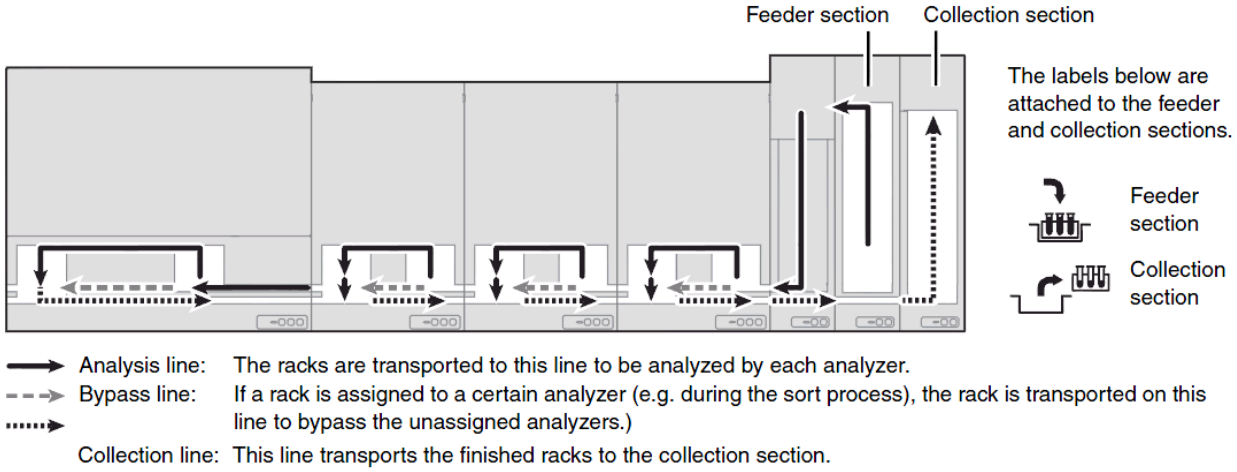
Running patient samples

Sample Requirements

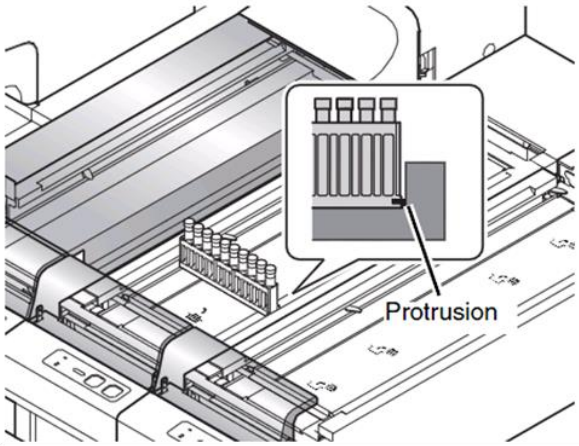
HbA1c analysis requires whole blood samples in EDTA tubes with a minimum blood volume of 1ml. Samples should be analyzed as soon as possible after collection. ***NOTE:*** *Gross lipidaemic samples my give erroneous results.*

System Mode

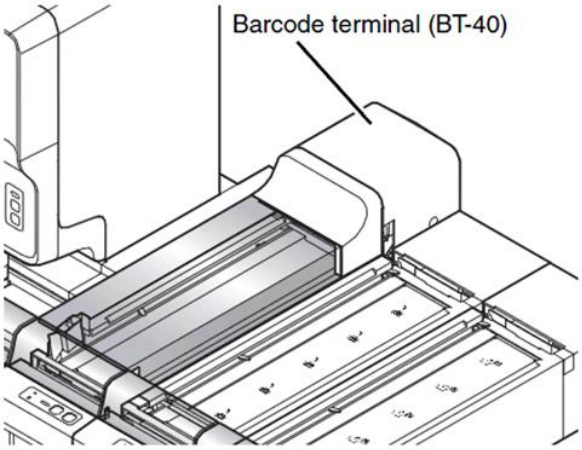
The location of the feeder section and collection sections can vary due to instrument combination. A typical example is shown below:



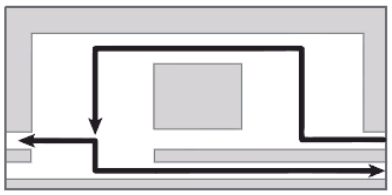
Load sample racks into the feeder section of the ‘Start Yard’ (ST). Automatic rack recognition will activate the rack pushers and the rack will be moved towards the back of the ‘Start Yard’ to be fed into the BT-40.

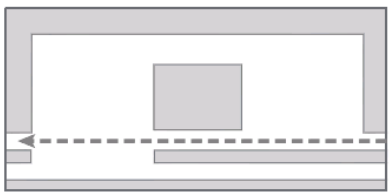


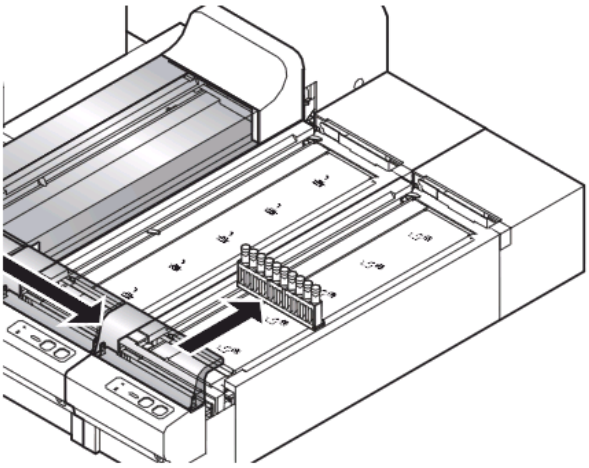
Racks will be moved into the BT-40 where sample barcodes will be read and the host computer will be interrogated to determine the analysis order for each sample number.



The sample racks are transported to the appropriate analyzer for analysis (top) or they bypass the analyzers not to be used (bottom).



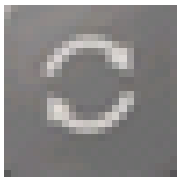




Following sample analysis the completed racks will be pooled in the stock yard where they can be removed or I present sent to the tube sorter for storage.

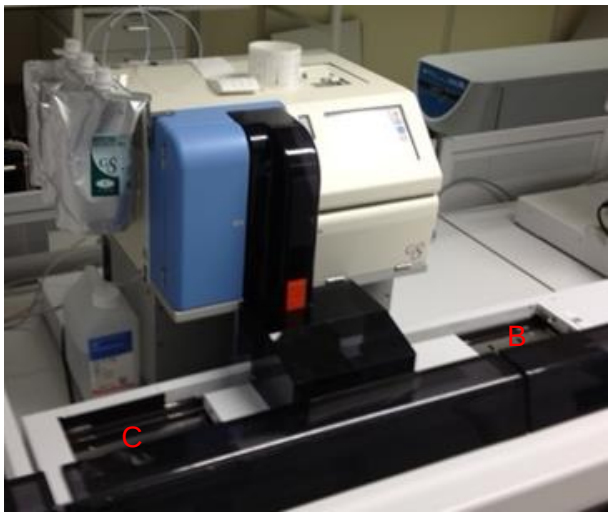
Sampler Mode

To place the TOSOH G8 in ‘sampler mode’ press the mode switch on the XN-track conveyor unit control panel. The indicator light will turn ORANGE. ***NOTE****: if running Calibrators, QC or pre diluted samples the TOSOH G8 will also need to be placed in Pre-dilution mode (See page 26).*



Racks can then be placed on the input area (B) of the conveyor unit. To start analysis press the [START] button on the TOSOH G8. Once aspiration of all samples in the rack is complete the rack will move to the output area on the left side of the conveyor (C).

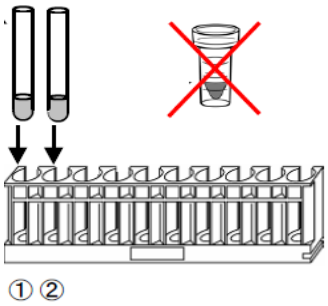
Once analysis is complete place the analyzer back into ‘system mode’ by pressing the mode switch on the XN-track conveyor unit control panel. The indicator light will turn GREEN.



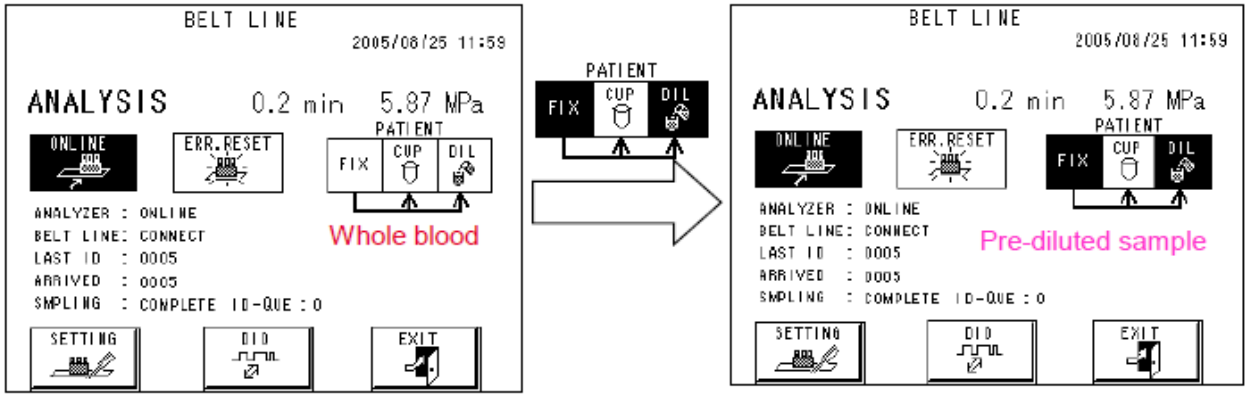
Pre-dilution Mode

Pre-dilution mode should be used for running Calibration material, QC and any samples less than 1ml. For QC preparation see page 21.

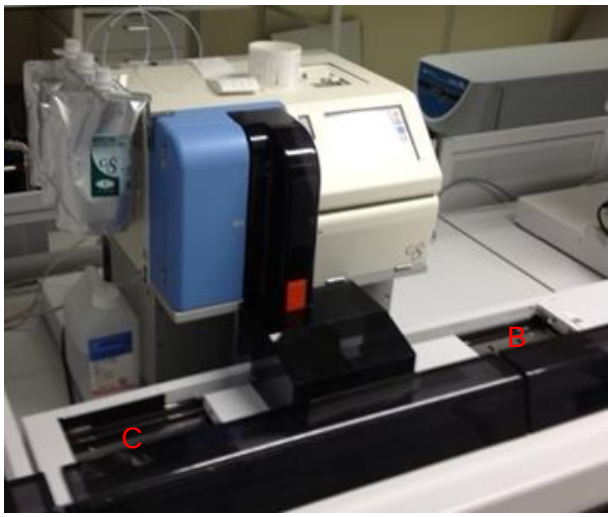
Patient samples requiring dilution should be diluted in a 1:200 dilution with haemolysis wash, 5µl whole blood to 1ml haemolysis wash. Samples should be placed in barcoded sample tubes and mix thoroughly before placing in the rack ***NOTE**: Samples can NOT be run in cups.*



To place the analyzer in pre-diluted mode from the 'Main menu' press [MENU] then [BELT LINE]. This will open the 'Belt line' screen, select [FIX] and [DIL].



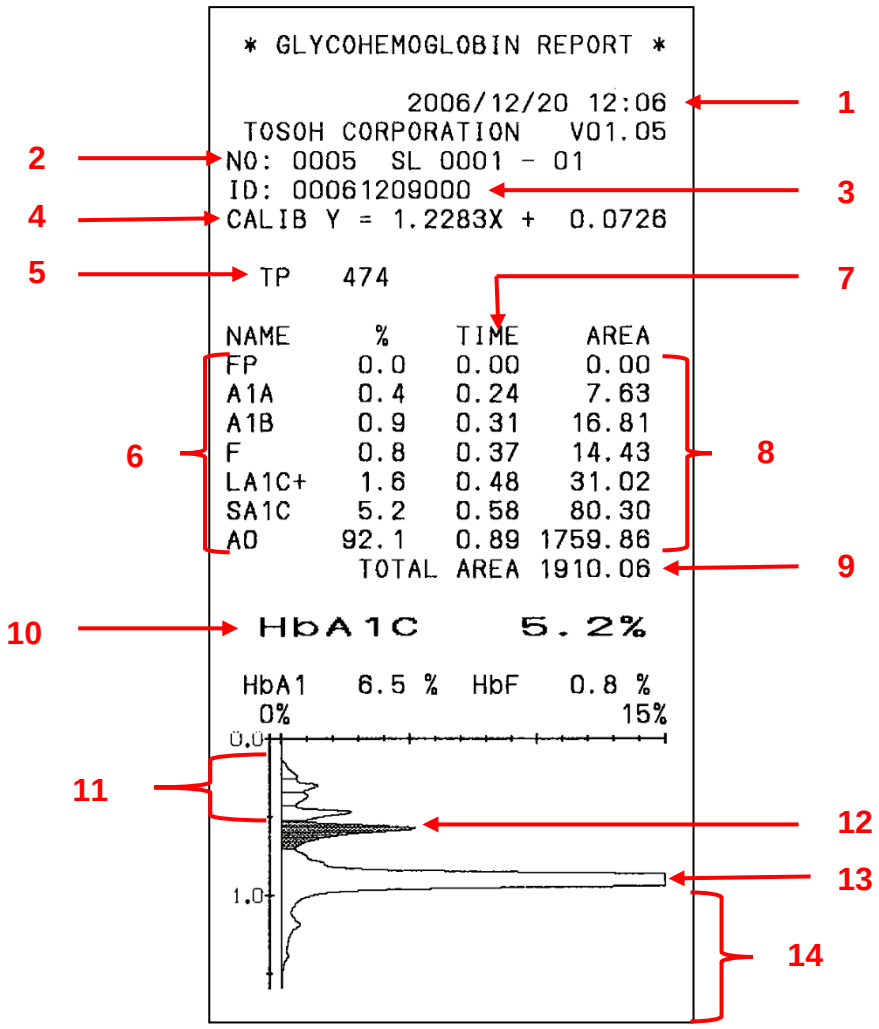
Racks can then be placed on the input area (B) of the conveyor unit. To start analysis press the [START] button on the TOSOH G8. Once aspiration of all samples is complete the rack will move to the output area on the left side of the conveyor (C).



Once analysis is complete place the analyzer back into 'whole blood' mode and back into 'system mode' (See page 25).

Chromatogram Interpretation

Normal Chromatograms



1. **Date and time of analysis** – displays date and time of analysis.
2. **Sample number of the day** – resets to one following startup of analyzer.
3. **ID** – displays the sample number or rack and position ID**.**
4. **Calibration equation** - Displays the calibration equation the HbA1c result is based on.
5. **Theoretical plate (TP)** - represents the quality of the calibration from the HbA1c peak. This figure must be greater than 250. A theoretical plate less than 250 indicates old column.
6. **Hb fractions** – lists the haemoglobin fraction in the order they are eluted within the column. Weakest fractions appear at the top of the list with the most positive fractions appearing at the bottom of the list.

7. **Retention time (RT)** - shows the retention time for the corresponding haemoglobin fragment eluted.
    - A1A 0.23
    - A1B 0.27-0.33
    - F 0.39
    - lA1c 0.47
    - sA1c 0.57 – 0.61
    - A0 0.88 – 0.92

    ***NOTE**: Retention times should NOT alter between reagent and column lots. RT errors are usually due to leaks, especially if the RT is late.*

8. **Area** – displays the corresponding area for each haemoglobin fraction detected.

9. **Total area (TA)** – sum of all the peak areas. Recommended range 700-3000. Acceptable range is 500-4000. ***Note:** Total areas outside these limits on one sample indicate sample problem. TA is out of range on multiple samples indicates analyzer problems such as blockage or dilution issue*.

10. **HbA1c %** - displays HbA1c percentage for that sample. Normal range is 20-42 mmol/mol (4.0-6.0%) with a target of <53mmol/mol (<7.0%) for diabetic patient.

11. **Hb Fragments** – peaks for different haemoglobins eluted with weaker binding to column resins than HbA1c, typically HbA1a, HbA1b, HbF, and LA1c.

12. **sA1c peak** – peak for the stable HbA1c (sA1c) fraction.

13. **A0 peak** – peak for haemoglobin A0 fraction.

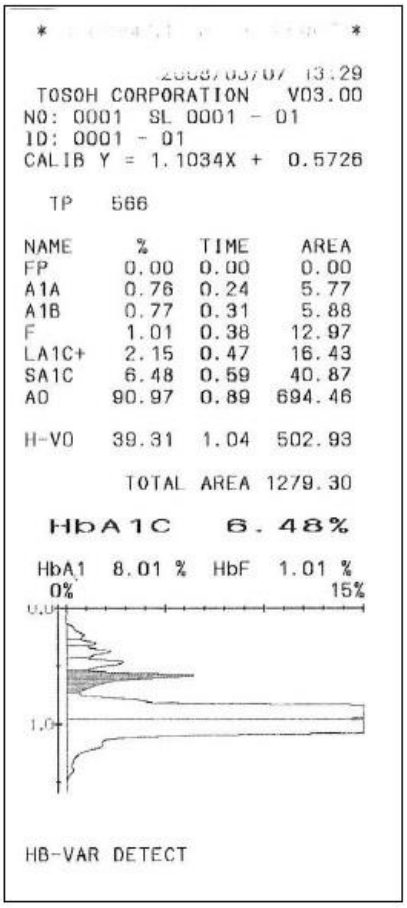
14. **Common Variant field (HbD, S and C) –** area where additional strongly bound haemoglobin variant fragment peaks will appear if present, resulting in an error flag (See page 29).

Chromatogram Flagging

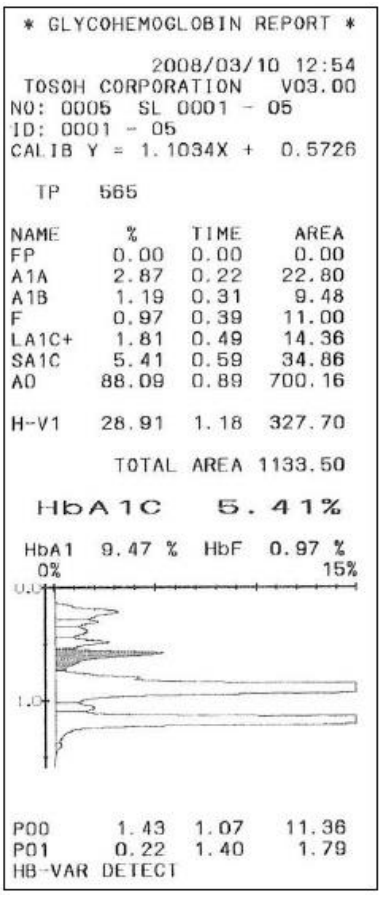
Three types of chromatogram flag can be seen

1. **HbF flag**: generated when HbF is >5%. HbF peak is automatically removed from the HbA1c peak.
    - HbF 5-15% results in a warning
    - HbF > 15% no result given

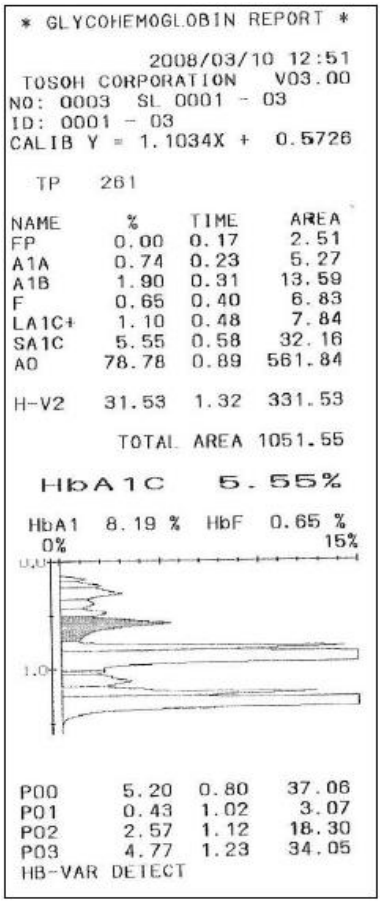
2. **Variant flags**: indicate the presence of a possible haemoglobin variant. The Hb variant peak is automatically removed from the HbA1c result. Results can be report with comment 'possible haemoglobin variant detected'. ***IMPORTANT:*** *Do NOT state which variant is present.* These results can NOT be used for diagnosis but can be used for monitoring.



H-V0: Possible HbAD



H-V1: Possible HbAS



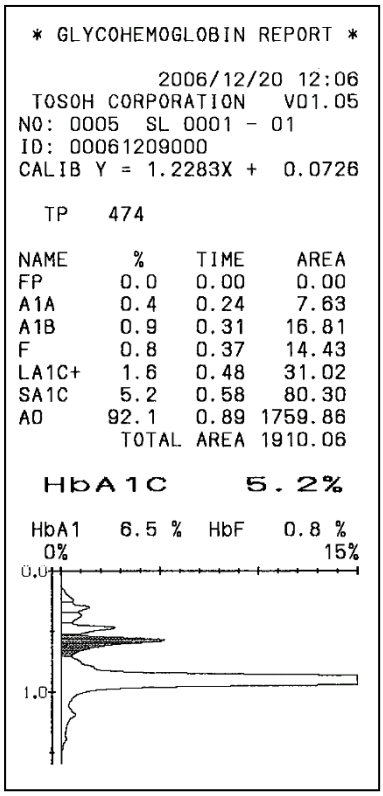
H-V2: Possible HbAC

3. **POO peaks**: unknown haemoglobin variants. POO peaks which appear after the A0 peak are ok to report. POO peaks before A0 with total percentage >5% invalidate HbA1c results and therefore should not be reported. These peaks could be due to other haemoglobin variant, glycated variant or possible old sample.

Checking Patient Results

When analyzing chromatograms (See page 27) the following points should be checked.

- Patient information
- The total area (TA) is within the recommended range. Recommended range 700-3000. Acceptable range is 500-4000. ***Note:*** *Total areas outside these limits on one sample indicate sample problem. TA is out of range on multiple samples (>3) indicates analyzer problems such as blockage or dilution issue*.
- The retention times (RT) for each fragment. sHb1c 0.57-0.61, A0 0.88 to 0.92.
- The theoretical plate (TP) value >250. ***NOTE:*** *As column ages TP will drop.*
- All peaks are represented, A1A, A1B, F, LA1C, SA1C and A0.
- Presence of flags HV or PO peaks (See page 29)
- HbA1c is within normal range
- Check for significant other fractions (See page 29)



```
 * GLYCOHEMOGLOBIN REPORT *

            2006/12/20 12:06
 TOSOH CORPORATION    V01.05
NO: 0005  SL 0001 - 01
ID: 00061209000
CALIB Y = 1.2283X +  0.0726

  TP   474

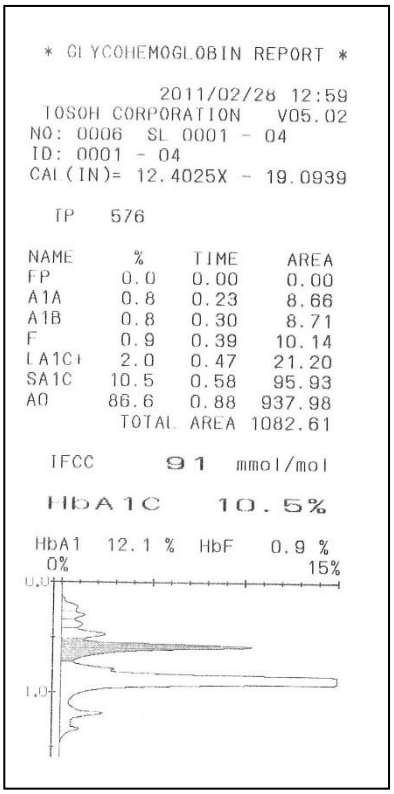
NAME      %     TIME     AREA
FP      0.0    0.00     0.00
A1A     0.4    0.24     7.63
A1B     0.9    0.31    16.81
F       0.8    0.37    14.43
LA1C+   1.6    0.48    31.02
SA1C    5.2    0.58    80.30
A0     92.1    0.89  1759.86
         TOTAL AREA  1910.06

HbA1C      5.2%

HbA1   6.5 %  HbF   0.8 %
 0%                   15%
```

Normal patient

- 6 Peaks
- TP>250
- RT sA1c ok
- Total area ok



```
 * GLYCOHEMOGLOBIN REPORT *

            2011/02/28 12:59
 TOSOH CORPORATION    V05.02
NO: 0006  SL 0001 - 04
ID: 0001 - 04
CAL(IN)= 12.4025X - 19.0939

  TP   576

NAME      %     TIME     AREA
FP      0.0    0.00     0.00
A1A     0.8    0.23     8.66
A1B     0.8    0.30     8.71
F       0.9    0.39    10.14
LA1C+   2.0    0.47    21.20
SA1C   10.5    0.58    95.93
A0     86.6    0.88   937.98
         TOTAL AREA  1082.61

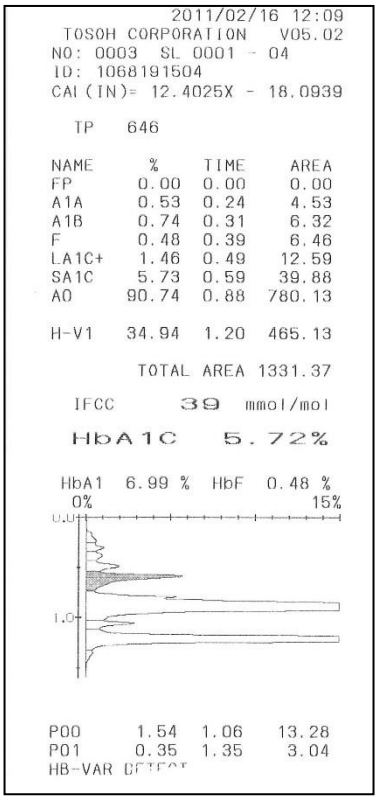
IFCC      91  mmol/mol

HbA1C     10.5%

HbA1  12.1 %  HbF   0.9 %
 0%                   15%
```

Diabetic patient

- Normal chromatogram
- HbA1c is higher than normal



```
                2011/02/16 12:09
 TOSOH CORPORATION    V05.02
NO: 0003  SL 0001 - 04
ID: 1068191504
CAL(IN)= 12.4025X - 18.0939

  TP   646

NAME      %     TIME     AREA
FP      0.00   0.00     0.00
A1A     0.53   0.24     4.53
A1B     0.74   0.31     6.32
F       0.48   0.39     6.46
LA1C+   1.46   0.49    12.59
SA1C    5.73   0.59    39.88
A0     90.74   0.88   780.13

H-V1   34.94   1.20   465.13

         TOTAL AREA 1331.37

IFCC      39  mmol/mol

HbA1C     5.72%

HbA1  6.99 %  HbF   0.48 %
 0%                   15%

POO     1.54   1.06    13.28
PO1     0.35   1.35     3.04
HB-VAR DETECT
```

Possible HbAS

- HV-1 peak 34.94%
- HbA1c is normal
- POO peaks present

In order to fully analyze results chromatograms should be studied in conjunction with patients full blood count. Confirmation of a haemoglobin variant can NOT be made from Hb Variant

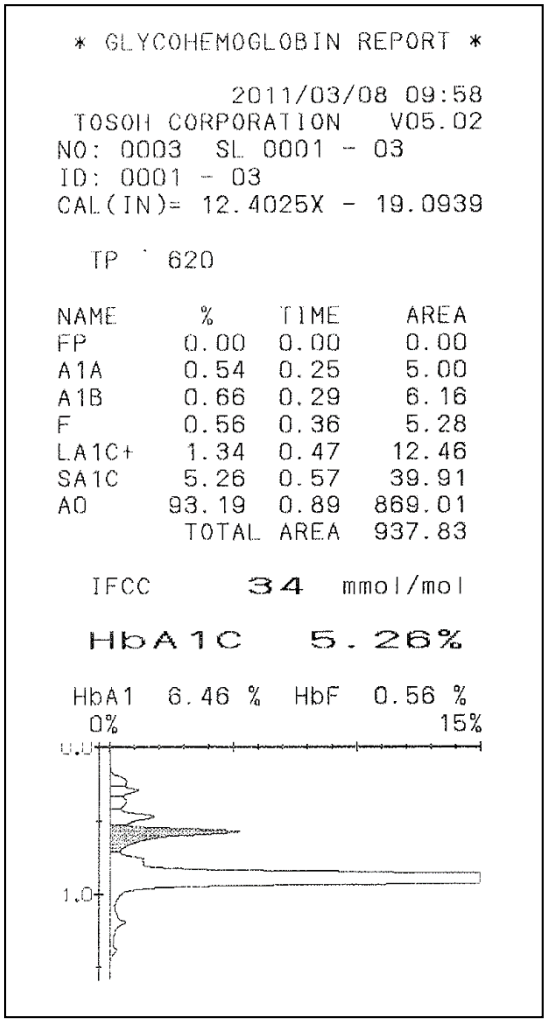
chromatograms. Variant identification can only be done by DNA analysis and any chromatogram in which such a variant is detected should be discussed with TOSOH.

Task 2: Chromatogram Interpretation

For each chromatogram below answer the following questions

1. Is the total area within the recommended range?
2. What is the retention time for HbA0 and sHbA1c?
3. What is the TP valve and is this acceptable?
4. Are all peaks present?
5. Are there any other significant fractions present?
6. Can the HbA1c value be reported? Are any comments required?

Chromatogram A



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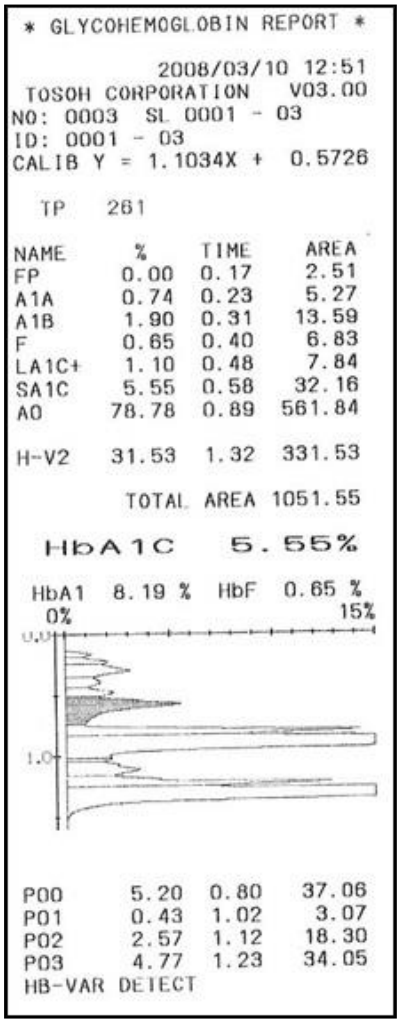
Chromatogram B



```
* GLYCOHEMOGLOBIN REPORT *

            2008/03/10 12:51
 TOSOH CORPORATION    V03.00
NO: 0003   SL 0001 - 03
ID: 0001 - 03
CALIB Y = 1.1034X +  0.5726

  TP   261

NAME      %      TIME     AREA
FP      0.00     0.17     2.51
A1A     0.74     0.23     5.27
A1B     1.90     0.31    13.59
F       0.65     0.40     6.83
LA1C+   1.10     0.48     7.84
SA1C    5.55     0.58    32.16
A0     78.78     0.89   561.84

H-V2   31.53     1.32   331.53

           TOTAL AREA 1051.55

   HbA1C     5.55%

HbA1  8.19 %  HbF  0.65 %
 0%                    15%
```



```
P00     5.20    0.80    37.06
P01     0.43    1.02     3.07
P02     2.57    1.12    18.30
P03     4.77    1.23    34.05
HB-VAR DETECT
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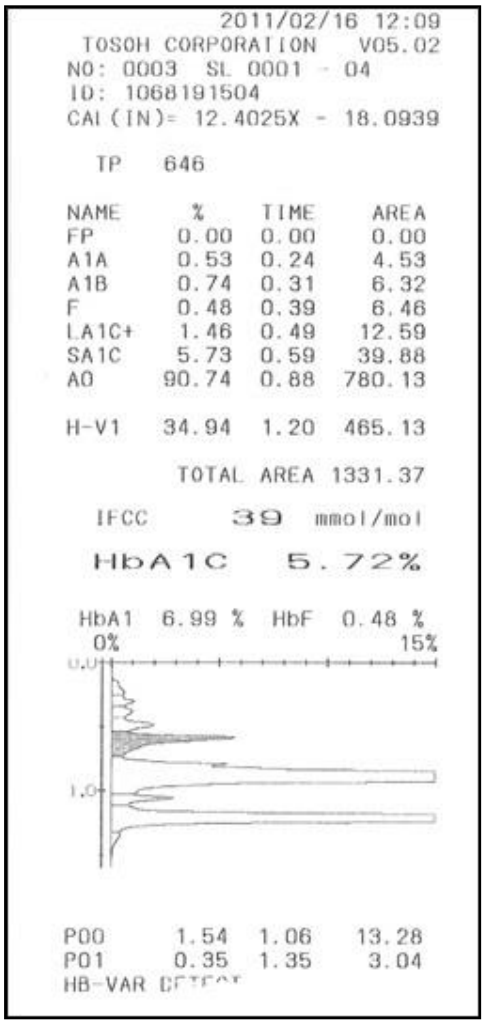
Chromatogram C



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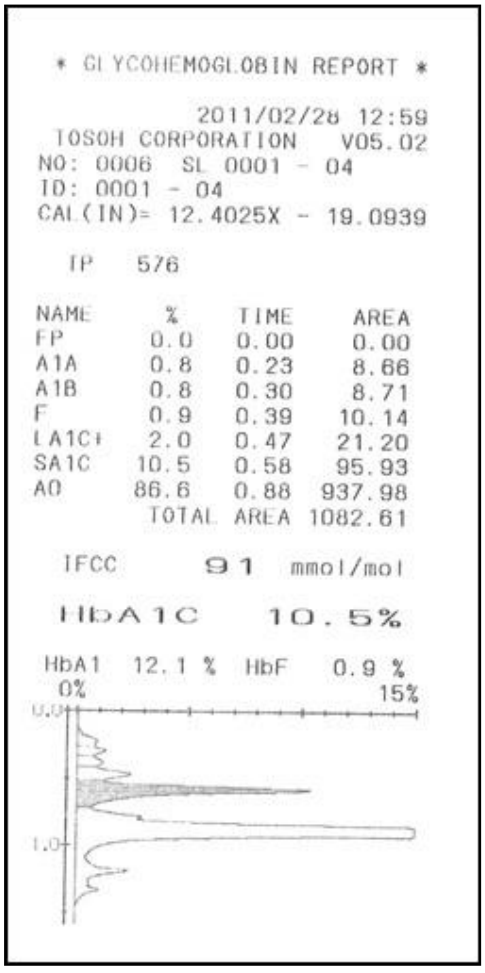
Chromatogram D



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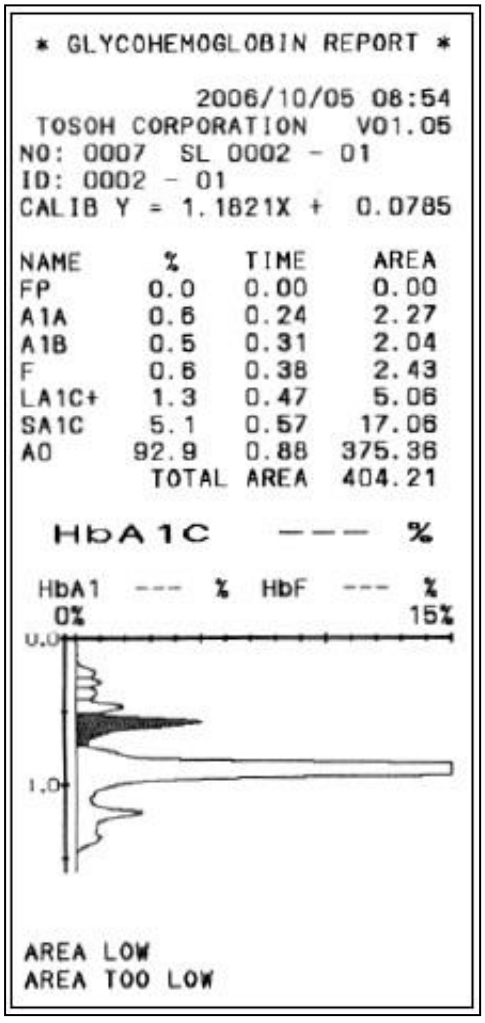
Chromatogram E



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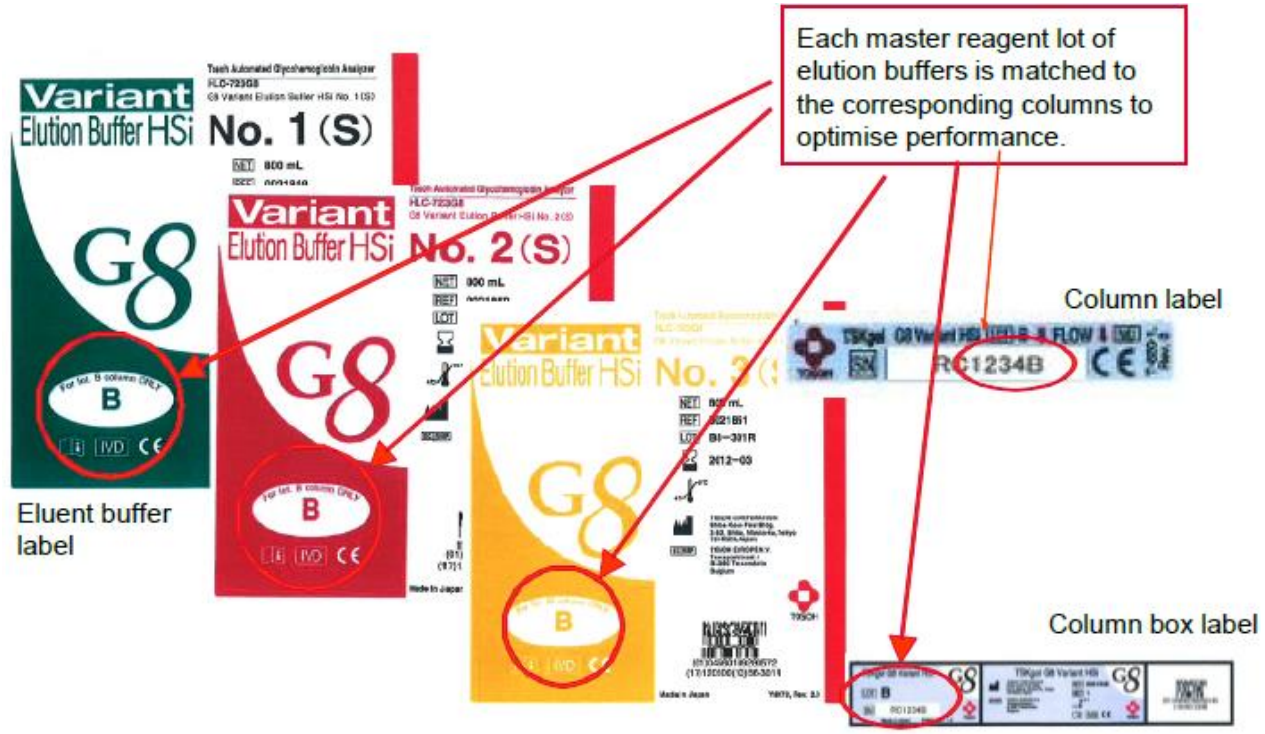
Changing Reagents

Reagents should be checked as part of daily maintenance and changed as soon as remaining levels are insufficient for next run. ***IMPORTANT****: there are NO level sensors on the reagent lines however the TOSOH G8 will alarm when buffer levels reach 3 – 5%. Failure to change reagent correctly will result in air being drawn into the analyzer.*

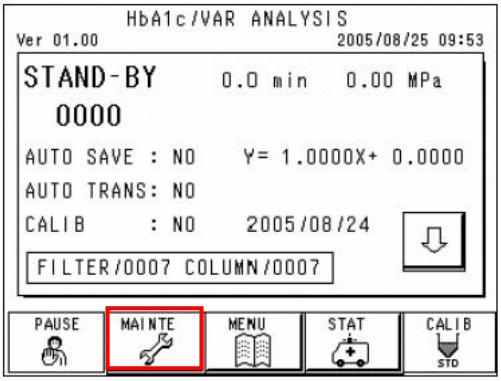
Before replacing the reagent ensure the analyzer is in 'STANDBY' and that the new reagent is the same lot letter as the column currently in use.

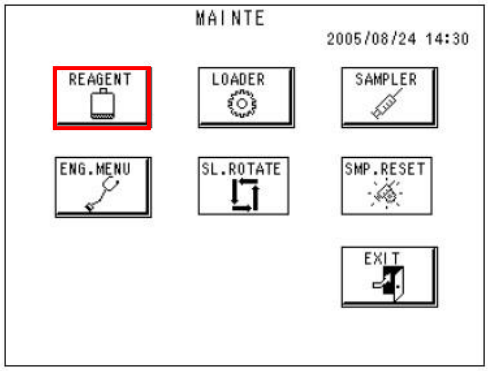


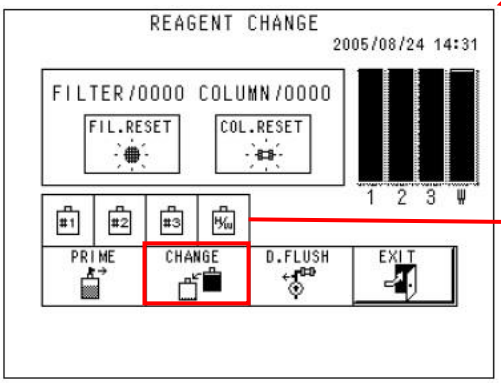
Remove the appropriate reagent container for the analyzer by unhanging reagent from reagent rack (Elution buffers only) and unscrewing the cap. Place the reagent probe directly into the new reagent container, ensuring the probe reaches the bottom and rescrew cap tightly. ***TIP****: if changing more than one reagent at a time ensure the right colour coded line is placed into the appropriate reagent container.* If changing a buffer place buffer back on reagent rack.

IMPORTANT*: Do NOT save or pool reagents. Discard any reagent left. Do NOT mix reagent lots.*

Once reagent has been replaced it must be registered on TOSOH G8. This can be done by entering the [MAINTE] screen from the 'main menu' screen then pressing [REAGENT].







Select appropriate reagent or reagents that have been changed. Press [CHANGE]. The following message will appear

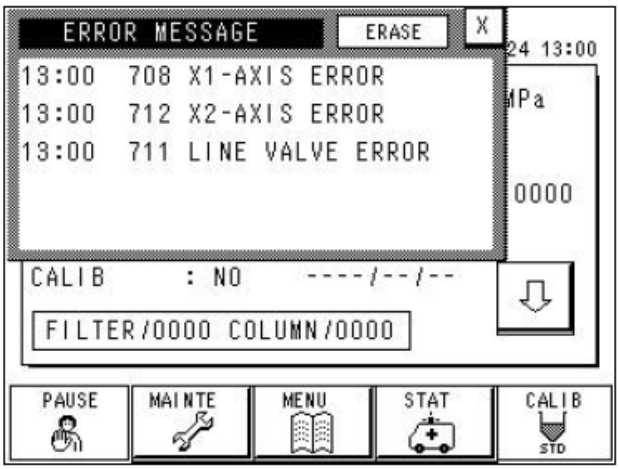
CHANGE --- EXECUTE?

Press [OK]. The reagents will then be primed replacing the reagent in the flow line.

***NOTE**: approximately 5ml of reagent is used during a reagent prime.*

Dealing with Errors

When a mechanical error occurs on the TOSOH G8 and alarm will sound and the error message will appear on the LCD screen. Press [E.RESET] to silence alarm and then deal with the error.



Troubleshooting

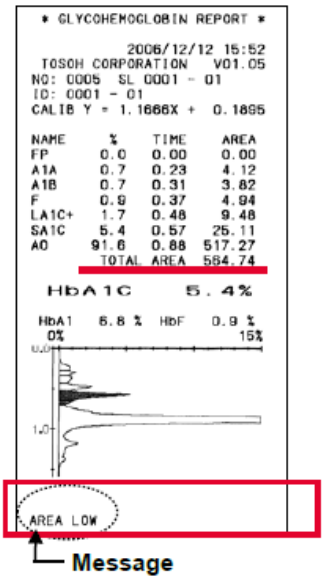
Area Too High/Area High

AREA TOO HIGH/AREA HIGH will appear as a ‘FLAG MESSAGE’ at the bottom of the result print out (See page 27). ‘AREA TOO HIGH’ is seen if the total area exceeds 4000 and the ‘AREA HIGH’ message is seen if the total area is between 3000 – 4000. The most common cause of this error is an inappropriately mixed sample or a high RBC count. Dilute the sample down using haemolysis wash solution to a 1:400 ratio.

Area Too Low/Area Low

‘AREA TOO LOW’ error is seen if the lower limit for the total area < 500. AREA LOW is seen if the total area reported is between 500-700. If this is seen on 3 successive results the analyzer will give an ‘ERROR MESSAGE’ which will appear on the TOSOH G8 LCD screen. The most common cause of this successive error is insufficient haemolysis wash. Check haemolysis wash and replace as necessary. Other causes of ‘AREA TOO LOW’ or ‘AREA LOW’ include column or needle blockage.

If ‘AREA TO LOW’ or AREA LOW are seen on individual samples the error message will appear as a ‘FLAG MESSAGE’ at the bottom of the result printout. This is most commonly due to insufficient sample volume. Check specimen volumes and if below 1ml dilute samples and run samples in ‘Pre dilution mode’ using a manual dilution 1:100 (See page 26).



Pressure High

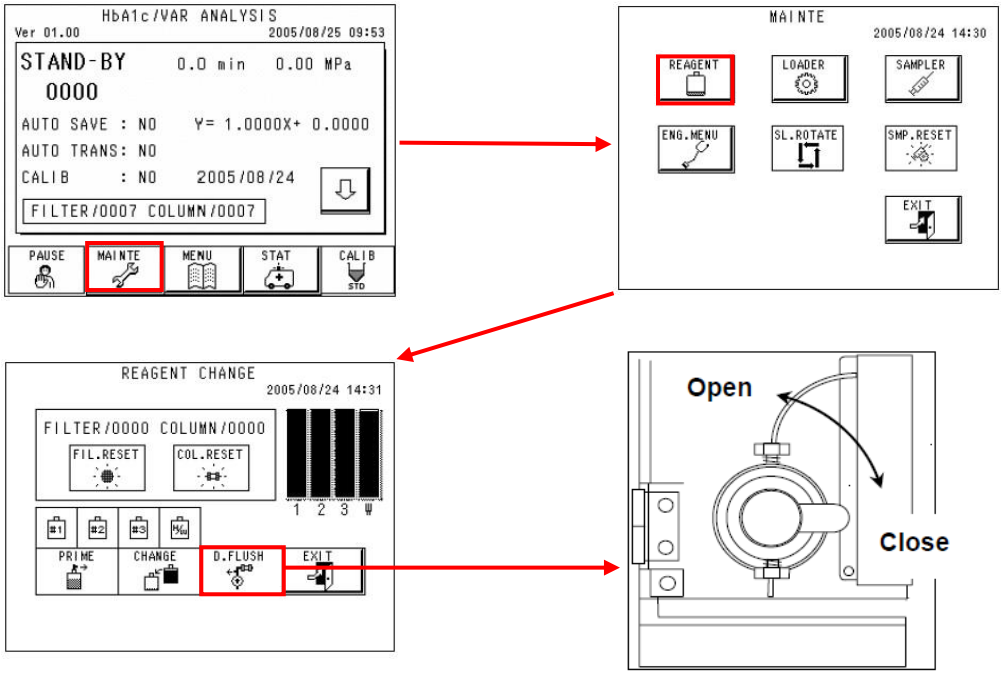
‘PRESSURE HIGH’ error is seen when the pressure exceeds the upper limit of 15MPa and results in the analysis of samples being stopped and the analyzer going into ‘STANDBY’. The most common cause is the filter or column replacement period has been exceeded. Alternative if filter count is less than 400 the filter maybe blocked.

Check filter count and column count on the main screen on the TOSOH G8. Columns are guaranteed for 3000 injections, however they can be run till between 5000-8000 injections as long as QC and chromatograms remain okay. The line filter should be replaced every 400 injection or when the pressure is +4 from the stated target (See column insert). Replace the column or filter as indicated by the appropriate counter (See page 17 and 16 respectively). If error continues contact your service provider.

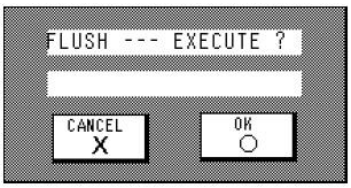
Pressure Low (Drain Flush)

'PRESSURE LOW' error is seen when air is present in the pump check valve. The most common causes of air entering the system is insufficient reagent(s) or leaks from either filter or column. Check reagent levels and replace as required (See page 37). Once reagent has been replaced perform a 'Drain Flush' (also known as pump air removal).

Ensure the analyzer is in STAND-BY, form the main screen select [MAINTE] screen and then [REAGENT] to enter the 'REAGENT CHANGE' screen. From here select [D.FLUSH] and the following message will appear 'OPEN DRAIN VALVE'. Open drain valve housing (See page 7) and turn the drain valve anticlockwise 90º degrees as indicated below and press [OK].

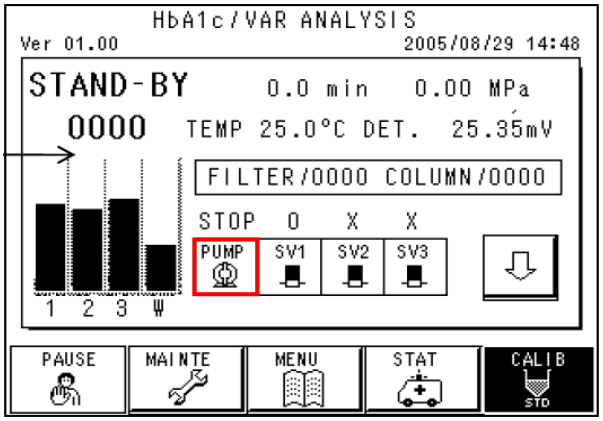


The following message will appear



Press [OK]. The drain flush procedure takes approximately 7 minutes and the air trap in the pump will be automatically removed. Once the procedure is complete the following message will appear 'CLOSE DRAIN VALVE'. Turn the 'drain valve back clockwise 90º degrees to the closed position as indicated above and press [OK]. Return to the main screen (Page 2) to perform a 'PUMP PRIME'.

A pump prime can be performed from by pressing [PUMP]. To stop the pump press [PUMP] again. Close column and filter housing cover.



Calib Error

'CALIB ERROR' occurs when the following conditions are met;

- The difference in sA1c % between the 2nd and 3rd calibration assays is > 0.3%
- The difference in sA1c % between the 4th and 5th calibration assays is > 0.3%
- The sA1c % result of any of the calibration assays 2, 3, 4 and 5 was ±30% of the assigned value.

Check the following points

- Check reagent lot letter and column lot letter match
- Check assigned value for calibrator lot
- Check chromatograms.
- Check calibrator dilution by repeat calibration ensuring dilution ratio is correct

Contact Us

Mail

Training Academy
Sysmex UK Ltd
Garamonde Drive
Wymbush
Milton Keynes
MK8 8DF

Phone

Product Hotline For urgent application support	0333 320 3466 (UK)
Service Hotline For technical support and service team	0333 320 3467 (UK)
Reagent ordering	0333 320 3470 (UK)

Email

Product mail (non-urgent)	productmail@sysmex.co.uk
Training Academy (non-urgent)	academy.training@sysmex.co.uk

Sysmex Academy

https://uk.sysmex-academy.com/

SCAN ME