Use of the TOSOH-GX for HbA1C in the Diabetes Centre

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Changes from last version of this document

Update to SHYPS format

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1 Purpose and Principle

The HLC-723GX is intended to assay HbA1C (% or mmol/mol) out of the total haemoglobin in blood for in vitro diagnostic use based on High Performance Liquid Chromatography principle with the cationic non-porous ion exchanger using the ionic difference. In addition to A1c (HbA1C), both HbA1 and haemoglobin F (HbF) can be measured.

Determination of HbA1C is used as a retrospective estimate of the average blood glucose level over a period of 8 to 10 weeks. Therefore, HbA1C determinations are long-term measurements of glucose metabolism. HbA1C is recommended as an essential indicator for the monitoring of blood glucose control. Include a short explanation of why we do the procedure or examination, i.e. the physiological basis / clinical relevance of testing and a description the chemical basis of the method.

2 Patient Preparation & Sample Requirements

Whole blood is used, taken directly from the patient by finger prick and diluted as described in section 10.1

3 Tasks, Responsibilities and Authorisations

Only staff who have undergone documented training as per training sheet PC-TEM-TSTOSOH are authorised to perform this procedure.

Trainee members of staff my complete the procedure under direct supervision by a fully trained member of staff.

4 Equipment

The analyser is situated in the laboratory of the diabetic clinic. The analysers are rented from Tosoh Bioscience (formerly Eurogenetics). The analyser is interfaced to a PC which is situated next to the laboratory analyser. This PC runs Tosoh's software for the handling and storage of chromatograms and results.

For technical support please phone 0333 320 3464. The TOSOHs are now under a managed service contract with Sysmex and this is their number. They will log the call and pass the information onto TOSOH. Preventative Maintenance takes place by TOSOH engineers on a three monthly basis.

Any downtime should be logged in the asset module in Q-Pulse against the appropriate asset.

For problems that are dealt with in house or are corrected with advice over the phone, please fill in an error log (LM-TEM-EQUIP ERR) and attach it to the asset in the asset module

If the problem requires an engineer visit, there is NO need to decontaminate the analyser. TOSOH say this is not required as the 'flush' cleans all areas (see CB-REC-TOSDECON).

Please run QCs (if the engineer does not) and complete an 'acceptance back into use' form (LM-TEM-EQUIP ACCEPT) after all engineer visits.

Please contact the Point of Care Team on ext. 5890 or Biochemistry on ext. 5802 with any faults.

Other consumables

Sample dilution cups for the TOSOH-GX are supplied by Starstedt. They are labelled 'Sample cup 2ml for Hitachi'.

Biohit tip 200ul pipette tips Cat No 790204 from Alpha Laboratory

Ordered by Senior in Special Chemistry when required

A small supply are kept at the clinic and topped up when required.

5 Chemicals and Reagents

The GX elution buffers are organic acid buffers and each contains less than 0.05% sodium azide as preservative.

GX Haemolysis and Wash Solution contain detergent and less than 0.1% sodium azide as preservative.

Stock reagents are stored at room temperature (4-30°) in the Point of Care Testing office. They are stable unopened until the expiry date on the bottle. Once opened they are stable for 4 months.

TOSOH Data Sheets can be found at https://www.diagnostics.us.tosohbioscience.com/msds/hplc.

Name	Classification & Specific Instructions
	Description: Preservative in Elution buffers and Haemolysis and Wash solution **Preparation:** N/A **Storage:** Room temp (15-25°) **Supplier:** Tosoh Bioscience Ltd Risk Statement & Control Measures This product is unlikely to cause harmful effects under normal conditions of use. Wear gloves and eye protection when handling stock solution. Action in the event of Spillage Clean with absorbent material. Dispose of absorbed material in accordance with LM-SOP-WASTMAN Hazard Identification & First Aid Measures  Toxic **Eye contact:** Open eyes as wide as possible and wash with clean water for at least 15 minutes. Immediately seek medical attention. **Skin contact:** Wash exposed area with plenty of soap and water. **Inhalation:** remove from exposure, rest and keep warm. **Ingestion:** Wash mouth with excess water and immediately seek medical attention. Liquid Waste Disposal Disposed as hazardous waste in accordance with LM-SOP-WASTEMAN

All reagents are supplied ready to use

Reagents for the TOSOH-GX come as a reagent assay kit (023161), which includes;

1x 800mL GX Elution Buffer No.1 (Green)
1x 800mL GX Elution Buffer No.2 (Red)
1x 800mL GX Elution Buffer No.3 (Yellow)
2x 2000mL GX Hemolysis & Wash Solution (Blue)
2x paper rolls
2x filter elements

TSKgel Variant Column (023160)
HbA1C G8 calibrator kit (0023526)
HbA1c Diluting Solution (0023503)

Always use the GX Elution Buffer with a TSKgel variant column of the same lot letter.

A standing order is in place and reagents and consumables arrive on a 3 monthly basis

If stocks are running low please inform the POCT coordinator.

6 Risk Assessment (Environmental and Safety Controls)

For full risk assessment please see PC-HSR-TOSOHGX

Risk	**Control measures**
COSHH – Reagents contain Sodium Azide	Wear gloves – staff to undertake training
Electricity – Potential Fire Hazard	Equipment PAT tested by estates
Infection – Danger of blood splash	Wear gloves – staff to undertake training
Sharps – Lancet use for capillary sampling	Use approved safety lancets – Dispose in sharps bins
Incorrect Result Reporting – Interpretation of chromatograms By non-laboratory staff	Staff to undertake training – Biochemistry to provide advice and interpretation for unusual patterns / error codes

Staff carrying out this procedure should have read and understood the Local Rules or Health and Safety Manual applicable to their site which should be followed at all times during the procedure.

This SOP and the associated risk assessment(s) have considered all hazards and necessary precautions required to control any risks identified. Where appropriate this is detailed in the COSHH assessment and Risk Assessment. Any risk; where possible is mitigated and or monitored with health surveillance to ensure health and safety for all those affected by this procedure

7 Calibration

The calibrators are supplied by TOSOH and are traceable to IFCC HbA1c Calibrator Set provided from IFCC Working Group on Standardisation of HbA1c.

A calibration is performed monthly BY POCT STAFF (as indicated on maintenance chart), or when control values assayed are out of range, after column replacement, after significant maintenance procedures e.g. plunger seal replacement, when a parameter value such as the flow factor is changed.

Material	Supplier	Cat Nº	Volume	Stability	Storage York
G8 HbA1c Calibrator Set (S)	Tosoh Bioscience UK	0023528	4 x 4mL bottles of each level (1) and (2)	Unopened: 2-8°C until expiry date on box Reconstituted: 30 mins at 30°C 8 hours at 2-8ºC	An in use box is stored in Fridge 29 bottom shelf Other stock can be found in Fridge T01596 on shelf 3

- Reconstitute both levels of calibrator by adding 1ml of the Tosoh HbA1c Diluting solution to each bottle of G8 HbA1c Calibrator.
- Rotate and/or invert calibrators thoroughly. Once reconstituted, calibrators may be used immediately.
- Press the CALIB key located at the bottom right of the main screen.
- The key is highlighted and the calibrator's assigned value input screen is displayed. Confirm the assigned value against the sheet provided and input the correct value if required. Press 'X' to exit and check that the CALIB key is highlighted.
- Put CAL (1) and (2) of the calibrator in sample cups, and set the sample cups in calibration holders No.1 (CAL1) and No.2 (CAL2), respectively. Press the START key.
- Once the automatic calibration is complete, the CALIB message will change to COMPLETED with the date of calibration and the CALIB key will no longer be highlighted.
- CAL (1) is the low value calibrator (approximately 6.0 %) and CAL (2) is the high value calibrator (approximately 10.8 %). The low value calibrator is assayed 3 times and the high value calibrator is assayed twice.

8 Quality Control

If the analyser is switched off, ensure the door is closed and then press POWER to turn on the machine. The analyser will carry out a pump clean which takes approximately 1 minute.

QC samples are supplied fresh weekly by the Biochemistry Lab and are kept in the fridge in the clinic laboratory next to the analyser.

1. QC samples should be poured into the corresponding barcoded dilution cups provided,
2. Place cups into positions 1 and 2 on the sample ring on the analyser with the barcodes facing out.
3. Close the door and press the start button on the top right of the analyzer.
4. After analysis complete, check the results are within range and record the results on the daily record sheet.

QC should be performed:

- At the beginning of each day
- After reagent and/or filter change;
- After calibration;
- After engineer repair;

9 External Quality Assurance (EQA)

The TOSOH GX is enrolled on UK NEQAS EQA scheme.

10 Procedural Steps

10.1 Obtaining a patient sample

- Gloves must be worn at all times
- Prepare a dilution cup by labelling with a sample barcode and use a pastette to dispense 1ml of haemolysate solution (blue) into the cup.
- Place an eye readable number matching the barcode used for the tube onto the Diabetic clinic worksheet (CB-TEM-DMCLINIC). A stock of these should be available at the clinic. Obtain from Q-Pulse if none are available at the location shown above.
- When the patient arrives sit them in the chair – **ask for and verify the patients' name and date of birth.**
- Place a patient sticky label onto the worksheet next to the eye readable number.
- Ask the patient which finger they would like the sample to be taken from. Prepare the stab site with a cotton wool swab soaked in water. Puncture the finger with the lancet. Draw 4ul of blood from the drop of blood using the Beckman fixed volume pipette. Use a clean tip for each patient.
- Give the patient a clean cotton wool ball and allow them to return to the waiting area.

10.2 Sample analysis

- Dispense the sample of blood into the numbered dilution cup. **Mix** the solution with the pipette tip. Discard the pipette tip and the used lancet into a sharps box.
- Place dilution cup into the sample ring, making sure all the barcode is visible.
- Close the door and press START.
- When the result is ready, check the chromatogram. If OK, enter the results on to the worksheet and into the patients electronic record (see section 8).

Remove the analysed sample from the Tosoh and place in the white rack until the end of clinic.

11

Reporting of Results

- **Check the Tosoh printouts for the following: -**

Look for any abnormal peaks. If you observe shoulders or splits around A1c or A0 peaks, the assay condition may not be optimum e.g. the reagents or column may have deteriorated. Please contact the Biochemistry department for advice.

Check for samples with any error flags such as: -

- Area High
- Area Low
- Hb Variant

Hb variant peaks usually manifest as a double A0 peak, after the HbA1C, and are accompanied by an 'Error 40, Variant' flag. Abnormally high levels of HbF (foetal haemoglobin) may be detected. This runs before the HbA1C and can sometimes merge with the 'LA1C' peak.

When **any** such abnormality is seen, first repeat the assay to check the result is correct and not due to a short or clotted sample.

If the chromatogram is the same on repeat please ring Biochemistry on ext 5802 and ask for the chromatogram to be checked before recording the results in the patient's notes, alternatively patient sample may be run on the alternative HbA1c measuring method available in Diabetes clinic.

Once the printout has been checked the HbA1c result printed in bold may be recorded on the result sheet and the patient's notes.

12 Reference Intervals

The following ranges are based on WHO recommendations;

- Non-Diabetics – 20-41 mmol/mol
- Impaired glucose regulation/Prediabetes – 42 – 47 mmol/mol
- Type 2 diabetes - >48mmol/mol

Reporting Range 20-140 mmol/mol

All daily, weekly and monthly maintenance and troubleshooting will be carried out by a member of the POCT staff. The instrument will be calibrated on a monthly basis or as required by Biochemistry/POCT. Record this maintenance on the daily tick sheet (PC-TEM-MAINTCTOS) as performed.

13 Maintenance

13.1 Changing reagents

The remaining volumes of buffers are displayed in a graph on the second screen by pressing the  key in the main screen (first screen).

Ensure the analyser is in Standby, before replacing the 3 buffers and the haemolysis solution (blue) as an entire assay kit. Make sure the tube reaches the bottom of the reagent pack and securely fasten the bottle caps to ensure a tight seal.

Press the 

key and highlight the keys of the reagents replaced, then press the 

key, then press ‘OK’.

13.2 Lifetime count

This is a monthly task and can be found under

- Menu
- Utility –
- Enter the password MAINT
- Exit x2
- Maint
- Engineer Menu
- Life count is visible mid screen

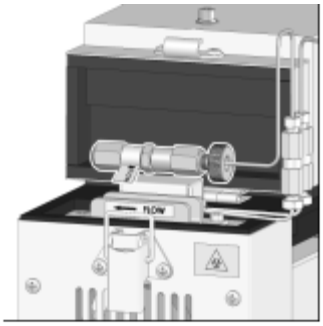
13.3 Other maintenance

Column replacement

Ensure the analyser is in Standby. Open the door below the screen, open the latch and remove the old column.

Remove the end fittings on the new column (do not discard).

Check the flow direction and fit the column to the right side first, with the flow label on the left.



Press the ⇩ key, confirm that the SV1 key is set to open (0) and SV2/SV3 keys are closed (x). This doesn't normally need changing, but if necessary - Press the keys to toggle between O and X until only SV1 is open.

Hold a tissue to the left side of the column to prevent fluid entering the analyser and press PUMP, when the solution begins to flow out of the left side of the column, press PUMP again to stop the flow.

Connect the left side of the column and then press PUMP. Ensure that the pressure stays within 0-+4 of the value stated on the column insert and that there is no leakage, then press PUMP to stop the flow.

Reset the column count on the REAGENT screen and take the 'column inspection report' to special chemistry to be stored in the 'Current Cal and QC' file.

Filter Replacement

Replace the filter when the filter count reaches 350 injections.

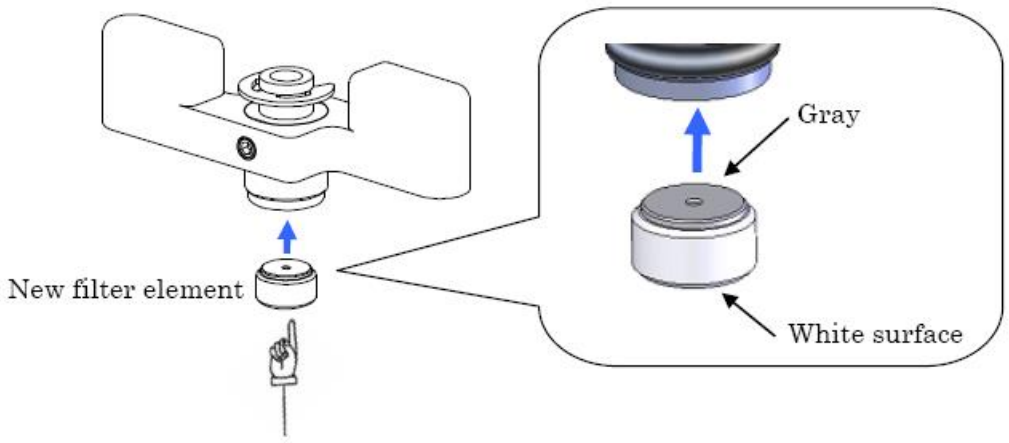
Ensure the analyser is in STAND BY and open the door below the display.

Confirm that SV1 key is open (0) on the second screen ⇩ and SV2/SV3 keys are closed (x). This doesn't normally need changing, but if necessary - Press the keys to toggle between O and X until only SV1 is open.

Unscrew the filter outlet (top) tubing.

Loosen top of the filter holder assembly by turning counter clockwise and remove the filter holder by pulling straight out.

Lightly press the top of the filter holder to take out the old filter element. Place the new element in to the holder. The grey coloured surface is the outlet (up) side.



Firmly tighten the top of the filter holder assembly by hand.

Place a tissue to the filter holder outlet and run the pump by pressing the PUMP key. This removes the air inside the element. Check no bubbles or there are no leaks coming from the outlet side. Press the PUMP key to stop pumping.

Re-connect the filter outlet tubing.

Press the PUMP key again to start elution buffer delivery. Confirm the pressure reaches 5MPa or more and that there are no leaks. Press PUMP key to stop pump.

Reset the filter count to zero on the REAGENT screen.

14 Performance Characteristics

Intra-assay precision

On installation intra-assay precision of s-A1c (mmol/mol) n=10

0.32%

Inter-assay precision

Internal QC values over a 20 day period:

Level 1 0.6%

Level 2 1.35%

Interferences

Very high HbF, the presence of HbE and other Hb Variants may affect the assay performance – chromatograms should always be interpreted with caution and a fresh sample run. If in any doubt, please contact POCT or send a sample to the laboratory for HbA1c confirmation.

15 Protocol for entering clinic results in the Laboratory

HbA1C's analysed in the Diabetic Clinic will be transmitted on completion, through the hospital network and appear on the GX Tab in the Piano software in the laboratory.

The clinic worksheet should be collected daily and returned to the POCT office.

Enter each patient into telepath via option 1 – Request Entry 1 – Registered patient/specimen

As the results have already been acted upon by the clinician during the clinic there is no requirement for hard copies of reports. Therefore, no consultant code needs to be entered in the request.

Location code - **YHDIA1-** *This code is only to be used for this purpose.*

Change date to date of analysis if results are not from today.

Report Comments - Initials of the operator from the Diabetes Centre (expands to their full name)

Clinical details – **D** (expands to diabetes mellitus)

Test code – **D** (expands to HbA1C).

Please type in RIO into report comment-extends to Request Intervention Override. This will stop TP or the DB from removing the HbA1c due to a previous result.

15.1 Results in piano

There are three worklist filters to distinguish result source, operated by four buttons at the bottom corner of the Piano software screen. Click GX to view clinic only results,

- All normal chromatograms shown in green. These can be released from Piano.
- Any chromatograms detected with a Flag will be held in **RED**
- Any sample that is released from Piano with an error code associated with it will hold in cITM. To release from cITM go to Overview-Samples pending release-Pick the correct sample from the display-Press release.

16 Related Forms/Templates and Documents

PC-HSR-TOSOHGX

PC-TEM-DIABWS

PC-SOP-EQA

17 References

The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-986.

TOSOH-GX Operator Manual