Use of the Roche B101 foe HbA1c Analysis

Document Author/Reviewer	Jane Mason
Document Owner	Jane Mason
Approved By	Rachel Lampard
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Changes from last version of this document

New SOP template

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1 Purpose and Principle

HbA1c is a fraction of haemoglobin which is used as an indicator of the average blood glucose concentration over the previous 2-3 months. It is therefore a good marker of diabetic control and HbA1c is the standardised measurement of longer-term control of blood glucose. The Cobas b101 Analyser is a portable analyser for the quantitative measurement of haemoglobin A1c (HbA1c) in whole blood at the point of care.

The blood sample is diluted and mixed with TRIS buffer to release haemoglobin from the erythrocytes. A fraction of the sample is conveyed into a reaction chamber where it is mixed with Sodium Lauryl Sulfate (SLS). SLS is used to form the SLS-haemoglobin complex. The concentration of total haemoglobin is calculated by measuring SLS-haemoglobin complex with a wavelength of 525 nm. Haemoglobin A1c (HbA1c) in another fraction of the sample is first denatured by the potassium ferricyanide and sucrose laurate. The denatured HbA1c bonds with HbA1c antibody on the latex particle. Latex agglutination inhibition reaction then occurs by reacting the agglutinator that has synthetic antigen which can bond with HbA1c antibody. The concentration of HbA1c is calculated by measuring the latex agglutination inhibition reaction with a wavelength of 625 nm. % haemoglobin A1c value is measured using a ratio of concentrations of HbA1c to total haemoglobin.

2 Patient Preparation & Sample Requirements

2µL fresh capillary whole blood or non-anticoagulated venous whole blood.

- All human blood samples must be treated as potentially BIO-HAZARDOUS.
- Approved Personal Protective Equipment (PPE) including lab coats, gloves and eye-protection must be worn when handling open blood samples or derivatives thereof.

	When performed according to the protocol detailed in this SOP, and in conjunction with adherence to Trust Policies and Good Laboratory Practice, the handling of patient samples represents minimal risk to staff.

Exposure to Bio-Hazardous Material

In the event of a needle stick injury or accidental blood splashes to eyes or mouth:

- If skin has been punctured encourage bleeding by gently squeezing. Wash with soap and running warm water then dry and dress the wound.
- Splashes to the eyes: irrigate eyes thoroughly with eye wash / saline
- Splashes to the mouth: gargle with drinking water (avoid swallowing)

Contact the Occupational Health Department / Emergency Department for guidance and report all adverse incidents to your line manager / complete a DATIX form.

Disposal of Patient Samples

No residual sample for disposal. Sample and Disk disposed of in Clinical Waste as a whole.

3 Tasks, Responsibilities and Authorisations

The system should only be used by staff members who have undergone training as per the training sheet PC-TEM-TSb101.

Training is documented in Cobas IT

4 Equipment

Roche Cobas b101 analyser

All equipment faults should be reported to the Point of Care Team on York ext.5890 or Scarborough ext. 2659.

5 Chemicals and Reagents

Test disks are stored at room temperature, the must be at 15˚C-32˚C for at least 20 minutes prior to use.

6 Risk Assessment (Environmental and Safety Controls)

The manufacturer indicates no particular hazard involved with use of the reagents.

GENERAL FIRST AID

The following first aid guidelines may be applied to all the substances detailed in this SOP.

Eyes: Irrigate thoroughly with water. At least 10 minutes is the recommended duration. Sterile saline is also available at the eye wash stations.

Lungs: Remove from exposure, rest and keep warm.

Skin: Wash substance off skin thoroughly with water. Remove contaminated clothing and wash before re-use.

Mouth: Wash out mouth thoroughly with water and give plenty of water to drink.

Remember – If at all concerned about the nature or severity of the problem, SEEK MEDICAL ADVICE.

For a full risk assessment please see PC-HSR-b101

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 analyser reads the lot specific calibration information from the bar code on each test cassette; no calibration is required by the end user.

This method has been standardized against the IFCC reference method for the measurement of HbA1c in human blood.

Each disc lot is traceable to IFCC.

8 Quality Control

Optical Check

The optical check is designed to check the optical function and accuracy of the entire instrument. For the test an optical disc is used. If the test passes successfully, the optical functions of the instrument can be considered to work normally.

The optical check should be performed in the following circumstances:

- Before the instrument is used for patient testing for the first time
- Before each clinic
- If a message for optical check lockout is displayed
- If an error message concerning the optical system is displayed
- If you want to check the optical function of the instrument
- After impact on the instrument (e.g. after accidentally knocking the instrument)

To perform the optical check:

- Log on to instrument using your Operator ID and Passcode.
- Choose Control Test > Optical Check. A screen is displayed asking you to open the lid.
- Choose Open, open the lid and load the optical check disc, and then close the lid.
    - The following error message may appear: Test cannot be performed due to improper disc placement.
      If the disc or turntable is soiled, the disc may not rest flat on the turntable. This may prevent the instrument from performing the test properly. To resolve this make sure the disc is free of soil and rests flat on the turntable.
- Once the test is complete a screen is displayed informing you whether the test has passed or failed.
- Choose ✓
- A screen is displayed asking you to open the lid. Choose ‘Open’, open the lid, remove the optical check disc and store it in its proper case.
- Close the lid.

Quality Controls

The Cobas b101 HbA1c control is a ready to use solution based on haemolysed human blood that comes in two concentrations, one within the normal range (level 1) and one within the pathological range (level 2).
Quality controls should be analysed in the following circumstances:

- Before using the instrument for patient testing for the first time

- When the quality control interval has been exceeded (weekly). When this point is reached the instrument will display a 'QC Lockout' message
- When using a new QC solution vial for the first time
- When using the first test disc of a new lot number
- If questionable test results are displayed repeatedly
- If you wish to test the performance of the instrument
- If a previous control test is out of range

QC Storage Conditions

Quality control samples should be stored at 2-8˚C.
Unopened, the control is stable until the expiry date stated. Do not use after the stated expiry date. Once opened, the control is stable for 7 days at 20-25˚C or 30 days at 2-8ºC provided that the dispensing of the control occurs without microbial contamination and when stored tightly capped. If there is evidence of microbial contamination or excessive turbidity in the product, discard the vial.

Procedure for running controls

- Log on to instrument using your Operator ID and Passcode
- From the main menu select Control Test > QC test
- A screen for choosing the control lot number is displayed. Checking the lot number on the QC solution that you are going to use, select the correct lot number.
- If you are performing QC for the first time, or if you are using a new QC lot number, select 'New QC Lot'
- A screen is displayed asking you to insert the QC info disc – ensure that you use the QC info disc contained in the control kit you are going to use
- Choose 'Open', open the lid and load the QC info disc, then close the lid. The test information is read into the instrument and is displayed on the screen
- Compare the control lot number on the bottle with that of your QC info disc – they MUST be identical
- Choose ✓
- A screen is then displayed for choosing a control level. Choose a level and then ✓
- Prepare the test disc
    - Ensure the test disc has been at 15˚C-32˚C for at least 20 minutes prior to use
    - Inspect the unopened foil pouch for tears and punctures – do not use a disc from a damaged pouch
    - Tear open the pouch at the notch end on the edge of the foil pouch
    - Remove the test disc from the pouch holding the hinge cover of the disc with thumb and forefinger, or thumb and middle finger - Do not touch the transparent surfaces of the disc.
    - The test disc must be used within 20 minutes of opening the pouch
    - Open the hinge cover fully – you will hear a click when the cover is fully open
- Apply the Level 1 solution
- Choose 'Open', open the lid and load the test disc, and then close the lid. A screen is displayed asking you to check the loaded solution.
- Check the information displayed on screen and confirm by choosing✓. If this is information is not correct you can abort the test by choosing X and confirming the abortion. The results are then displayed as 'Aborted'.
- The test is performed. When it is complete, a screen is displayed asking you whether you want to proceed with the QC solution of the other level.

- Choose ✓ to confirm that you want to test the other level as well. A screen with instructions is displayed.
- Prepare the disc
- Apply the Level 2 solution
- Repeat the process as above
- When the test is complete, Pass or Fail information will be displayed. Results are stored in the instrument according to the date and time.
- If your QC consistently fails please contact the Point of Care Testing department on York ext 5890 or Scarborough ext 2659.

9 External Quality Assurance (EQA)

Current scheme WEQAS

EQA samples differ from internal quality controls in that the accuracy of the procedure is not known until after the results have been issued. As the user does not know the HbA1c result at the time of analysis, and the results are assessed independently, it allows confidence that the performance of the analyser and operators is not varying over time.
Samples are issued from Biochemistry. The samples should be tested on receipt, and the results returned on the form provided, as soon as possible. If analysis cannot be performed immediately store the samples in the fridge until analysis can take place. Best performed within 2 days of receipt.

10 Procedural Steps

Running patient samples

1. Ensure the test disc has been at 15˚C-32˚C for at least 20 minutes prior to use
2. From the Main Menu on the instrument select ‘Patient Test’
3. You will be asked to provide an operator ID - scan your operator ID barcode.
4. Enter patient NHS as ID.
5. When the instrument is ready to perform the test you are asked to prepare the disc and open the lid
    a. Tear open the pouch at the notch on the edge of the foil pouch.
    b. Remove the disc from its ouch and place it on a clean surface – make sure not to touch the suction point or the transparent surfaces.
6. Generate a good blood sample.
    a. Wash and thoroughly dry the patient’s hands.
    b. Immediately after lancing the finger massage gently along the finger to obtain a good blood drop without squeezing or pressing. Wipe away the first blood drop, as this may contain tissue fluid. Gently massage the finger again until a second large blood drop forms
7. With the front of the test disc facing upwards position the discs suction point above the blood drop
8. Apply blood
9. Check that a sufficient sample volume has been applied. Turn the disc over – the area marked in blue has to be completely filled with blood.
10. Close the disc by pressing the hinge cover down firmly.
11. Open the lid of the instrument.
12. Place the disc on the rotor – make sure the printed side of the disc is facing upwards and do not touch the transparent surfaces.
13. Close the lid. The instrument will automatically start processing the test.

14. Once the test is complete you will be prompted to accept the result before being able to remove the disc.
15. Dispose of used disc in clinical waste.

11 Reporting of Results

- Results are displayed on screen when the system finishes analysing the sample. The screen displays result values and demographics for each test.
- The HbA1c results from the analyser are displayed in mmol/mol. (IFCC calibration). The working range is 4 -130 mmol/mol.
- If a patient result is outside of the permissible ranges a message is displayed indicating the range and whether the results is above [Hi] or below [Lo] the range.
- If any other messages are shown, refer to the operating manual.
- Results are automatically transferred the patients electronic record, but you are still required to document them as directed by the clinic lead.
- Results are also stored on the instrument and can be viewed by following the steps below:
    - From the Main Menu choose Review Results > Patient Results
    - The Patients Results screen is displayed
    - Choose ‘HbA1c’
    - A list is displayed with a button for each result. Selecting a result allows you to see all of the demographics and information associated with that result

If any result appears questionable or if the clinical signs and symptoms appear inconsistent with the result, re-test the patient and / or confirm the result by sending a sample to Biochemistry.

12 Reference Intervals

Non-Diabetics 20 to 42 mmol/mol

This is a historical reference range used within this laboratory.

The upper limit of this reference range was amended in accordance with the dialectologists in line with The Diabetes Control and Complications Trial Research Group. 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.

13 Performance Characteristics and limitations

Any cause of shortened erythrocyte survival will reduce exposure of erythrocytes to glucose with consequent decrease in mmol/mol HbA1c values (IFCC) even though the time-averaged blood glucose level may be elevated. Causes of shortened erythrocyte lifetime might be haemolytic anaemia or other haemolytic diseases, homozygous sickle cell trait, pregnancy, recent significant or chronic blood loss, etc. Caution should be used when interpreting the HbA1c results from patients with these conditions.

Glycated HbF is not detected by the assays it does not contain the glycated beta-chain that characterisesHbA1c. However, HbF is measured in the total Hb assay and as a consequence, specimens containing a high amount of HbF (>10%) may result in a lower than expected mmol/mol HbA1c values (IFCC)

If the concentration of haemoglobin is less than 60 g/L, or higher than 200 g/L, no test result is reported.

Interferences

Icterus: No significant interference up to a conjugated/unconjugated bilirubin concentration of 1000umol/L / 60mg/dl

Lipaemia: No significant interference up to an intralipid concentration od 500 mg/dl

Glycaemia: No significant interference up to a glucose level of 111mmol/L

Rheumatoid factor: No significant interference up to a Rh Factor level of 750 IU/ml

Drugs: No interference was found at therapeutic concentrations using common drug panels.

14 Related Forms/Templates and Documents

PC-HSR-B101

PC-SOP-B101

15 References

Roche Cobas b101 System Operators Manual