National Standard Operating Procedure of Bio-Rad Variant II HPLC Analyzer Maintenance and Operation

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Acronyms

MOH	Ministry of Health
DGHS	Director General of Health Services
SOP	Standard operative procedure
HPLC	High Performance Liquid Chromatography
IQC	Internal Quality Control
EQC	External Quality Control
QC	Quality Control
P & P	Policy and Procedure
DI water	Distilled water
Hb	Hemoglobin
CDM	Clinical Data Management
VSS	Variant II Sampling Station
VCS	Variant II Chromatographic Station

1. **Purpose:**

   This standard operating procedure (SOP) document will provide a guide on the operation and maintenance of the Bio-Rad Variant II HPLC Analyzer.

2. **Scope:**

   This document is applicable to all laboratory technologists (MOH-Oman).

3. **Definitions:**

   - Bio-Rad Variant II HPLC: An automated system used for analyzing hemoglobin variants using high-performance liquid chromatography.
   - High-Performance Liquid Chromatography (HPLC): is an analytical technique used to separate, identify, and quantify individual components in a mixture.

4. **Procedure**

   4.1 Clinical background:

   High-Performance Liquid Chromatography (HPLC) is a cornerstone of modern clinical diagnostics, particularly for the identification and quantification of haemoglobin variants. Hemoglobinopathies are a group of inherited genetic disorders affecting the structure or production of the haemoglobin molecule such as sickle cell disease and thalassemia's. These conditions represent a significant global health burden, and their accurate diagnosis is crucial for effective patient management, genetic counselling, and neonatal screening programs.

   The system's ability to provide a quantitative analysis of common hemoglobins such as HbA, HbA_2, and HbF, as well as detect a wide array of clinically significant variants like HbS, HbC, HbE, and HbD, makes it an invaluable tool. The resulting chromatogram offers a distinct "fingerprint," where each hemoglobin variant appears as a peak at a characteristic retention time. This high degree of resolution and reproducibility supports its status as a gold-standard method for both primary diagnosis and confirmatory testing, complementing other methods like electrophoresis and genetic analysis.

   4.2 Principle:

   The Bio-Rad™ VARIANT™ II Haemoglobin Testing System is a fully automated analyser that utilizes the principle of cation-exchange HPLC. In this technique, a sample of hemolysate is injected into a column packed with a stationary phase of negatively charged particles. A mobile phase with a gradually increasing ionic strength is then pumped through the column. Haemoglobin variants, which differ in their amino acid composition and therefore their overall charge, interact with the stationary phase

to varying degrees. Molecules with a more positive charge bind more strongly and are eluted later than those with a weaker positive or net negative charge. A detector measures the absorbance of the eluate at 415 nm, the peak absorbance for heme, allowing for the precise quantification and identification of different haemoglobin fractions based on their specific retention times.

4.3 Pre – analytical stage:

4.3.1 Specimen requirements and preparation:

4.3.1.1 Sample type: whole blood in EDTA a minimum of 1.5 ml. Storage up to 7 days at 2-8°C. 48 hours stable if stored at ambient temperature (22-24°C).

4.3.1.2 Each request must be handled on a one-by-one basis. Check patient details (name, patient ID and specimen ID) on the specimen which must match the details on the electronic request.

4.3.1.3 Transportation: in a specimen bag labeled as biohazard.

4.3.1.4 Specimen should be free from any clot, fibrin clot or hemolyzed sample

4.3.1.5 Do not pre-dilute the sample unless the blood volume is less than 1.5 ml. If the blood volume is less than 1.5 ml, then pre-dilute the sample 1:200 in a 1.5 ml vial (5 ul sample to 1 ml wash diluents).

4.3.1.6 When the total area of an analysis is out of the standard voltage between 1 million to 3 million. The following must be done:

- Sample analysis less than 1,000,000 should be manually diluted as follows:
  - 1:150 dilution factor by adding 1.5 ml wash diluents to 10 µl blood sample
  - (or) 1:100 dilution factor by adding 1.0 ml wash diluents 10 µl blood sample (when required).
  - If the area still less than 1,000,000 then centrifuge the sample and take from the packed RBC 5 µl blood into 2 ml wash diluents (If the color is pale, then add 10 µl blood instead of 5 µl blood)
- Sample analysis greater than 3,000,000 an increase of dilution factor is required by manually mixing:
  - Dilution 1:400 (5 µl of blood sample in 2 ml wash diluents)

4.3.2 Reagents: (see appendix 1 for preparation)

4.3.2.1 **Whole blood primer:** Reconstituted primer is stable for 21 days when stored at 2-8 C.

4.3.2.2 **HbA2/F Calibrator:** Stable for 10 days at 2-8°C.

4.3.2.3 **Controls:** Suggest running controls at the beginning of the run. If the sample number is expected to exceed 50 samples in one run , consider running the control twice. It is stable for 21 days at 2-8°C.

4.3.2.4 **Blank:** Contain Distilled Water.

4.3.2.5 Elution Buffer 1 and 2 stability 60 days after opening.

4.3.2.6 Wash/Diluent solution stability 60 days after opening.

4.3.3 Materials:

4.3.3.1 Bio-Rad Variant II Analyzer.

4.3.3.2 Precision pipettes

4.3.3.3 Sample vials

4.3.3.4 Analytical cartridge

4.3.3.5 CD-ROM

4.3.4 Daily maintenance:

4.3.4.1 Pre-run checklist **(see Appendix 2)**: Check that the correct method is installed. Check the buffer/wash levels, lot numbers and line positions.

4.3.4.2 Check the cartridge injection count. Check the pump pressure on both pumps.

4.3.4.3 Flow rate should be programmed according to the installed kit (B-Thal runs at 2 ml/min). The expected pressure range for each cartridge lot is listed in the cartridge inserts (pressure averages 18-25 kg/cm). And Pumps should not fluctuate more than 5%.

4.3.4.4 Check for leaks during pressure check. And check the waste container level.

4.3.4.5 Check printer paper supply.

4.3.4.6 Perform piston seal flush.

4.3.4.7 Check control 1 and 2 and run it.

4.3.4.8 Maintenance Daily log sheet should be signed by the scientist.

4.3.5 Monthly maintenance (see Appendix 2):

4.3.5.1 Clean Exterior/Interior surfaces/Belts, Clean barcode reader, Clean and inspect sample racks.

4.3.5.2 Clean and Decontaminate the Sampling Fluid Path:

4.3.5.3 The decontamination procedure should be performed once per month. Disconnect the cartridge-to-detector tubing from the cartridge holder.

4.3.5.4 Remove the analytical cartridge and rinse the cartridge holder with DI water.

4.3.5.5 Install the PEEK dummy cartridge into the holder. Then install the decontamination tubing onto the outlet of the cartridge holder. Place the outlet side of the decon tubing into a beaker to collect waste from the decontamination procedure.

4.3.5.6 Select the **V2_DECON** test from the **Setup/Test** screen. **Current Test: V2_DECON, Reagent Set: Default**, Click **OK** on the Test dialog box.

4.3.5.7 Place 5 sample micro vials filled with 5% sodium hypochlorite solution (undiluted household bleach) into adapters in the first 5 positions of a sample rack.

4.3.5.8 Place 5 sample micro vials filled with DI water into adapters in the last 5 positions of the sample rack.

4.3.5.9 Prepare 5 mL of a 1:10 dilution of whole blood in DI water (i.e., 500 µL of whole blood in 4.5 mL of DI water). Mix thoroughly. Place 5 sample micro vials, containing 1 mL each of the 1:10 hemolysate solution, into adapters in the first 5 positions of a second sample rack.

4.3.5.10 Place the STOP adapter with an empty sample micro vial into position 6 of the second sample rack. Place both sample racks on the VSS. And Start the Work list.

4.3.5.11 When the status returns to Ready, remove the PEEK dummy cartridge. Rinse the cartridge holder again with DI water. Reinstall the original or new analytical cartridge.

4.3.5.12 Remove the decon tubing from the cartridge holder. Discard the beaker waste. Reconnect the cartridge-to-detector tubing, securing both tubing end connections.

4.3.5.13 Select the desired test from the **Setup/Test screen.**

4.3.6 Cartridge Handling: -

4.3.6.1 Store at 15-30 °C For all new cartridges (0 injections) a double priming and cartridge temperature adjustment must be performed.

4.3.6.2 Use Update Kit CD via Setup/Test screen. Insert Update kit CD-ROM into CD driver, Click Update kit, select drive e: Select test to be updated (V2_bthal). And Click OK.

4.3.6.3 Install the cartridge in the direction indicated by the arrow on the cartridge.

4.3.7 New cartridge: priming and temperature adjustment:

4.3.7.1 Place the cartridge into the cartridge holder and position the cartridge holder in the thermo-module; close and secure the thermo-module and place the following in the sample rack:

Tube position	Adapter label	Reagent
1	Primer	Whole blood primer
2	Primer	Whole blood primer
3	Blank	DI water
4	Blank	DI water
5	Blank	Variant A2/F Calibrator
6	Calibrator	Variant A2/F Calibrator
7	Stop	

4.3.7.2 Verify that the system is in ready state and go **to the run/work** list screen and press the '**start/stop**' icon to initiate the run.

4.3.7.3 Once the run is finished, note retention time of A2 from tube 6. Refer to releasing notes provided in the kit for the optimum A2 retention time (RT) and use the chart to make the appropriate temperature adjustment, if any is needed.

4.3.7.4 Adjustment temperature for cartridge according to the following table:

RT of HBA2 in Calibrator	Temperature Adjustment
3.53	-5
3.55	-4
3.58	-3
3.6	-2
3.63	-1
3.65	0
3.68	+1
3.7	+2
3.73	+3

4.3.8 Quality Control:

4.3.8.1 Internal Quality Control: Normal (HbF, HbA2). Abnormal (HbF, HbA2).

4.3.8.1.1 IQC monitoring and limit calculation:

1. Initially use manufacturer range and mean for 20 points. Then establish lab range by calculating new mean, SD, CV%. It should fall within the manufacturer range.
2. Check the measurements, as they must be within the mean and 3 SD. If any measurement is out of the range,(check the lab range if reading within lab range then run the sample if not) then the outlier should be rejected, and re-calculation of mean and SD must be done.
3. Record the IQC results in the internal QC record form.
4. Monitor QC by monthly CV% which should be < 7%.

4.3.8.2 **External Quality Control (EQC):** Follow the instruction according to the external quality program which is provided by MOH.

4.3.9 Safety precaution:

4.3.9.2 All specimens need to be treated as potentially infectious.

4.3.9.3 Standard procedures for handling biohazard material must be followed at all times.

4.3.9.4 Universal Precautions must be practiced at all stages of these procedures.

4.4 Analytical stage:

4.4.1 Routine sample run:

4.4.1.1 Place the following in the sample rack:

Tube position	Adapter label	Reagent
1	Primer (PR)	Whole blood primer
2	Blank	DI water
3	Blank	Variant A2/F Calibrator
4	Calibrator	Variant A2/F Calibrator
5	Control level 1	Low Control
6	Control level 2	High Control
7 to N	Patient Samples	
N+1	Stop	

4.4.1.2 Place the sample rack onto the VSS belt, verify that the system is in ready state. Go to the **run/work list** screen and press the '**start/stop'** icon to initiate the run.

4.4.1.3 Ensure data back-up and result validation - Maintain maintenance and QC logs (Daily and Monthly).

4.4.1.4 The data and results should be backed up annually in CD-ROM.

4.5 Post – analytical stage:

4.5.1 The reporting of HPLC and interpretation (Refer to HPLC Result Interpretation for Hemoglobin Analysis SOP)

5. Responsibilities:

5.1 Laboratory Technologist:

- Operate instrument, perform maintenance, run and review QC/calibrators.
- Document and store all specimens properly.
- Adhere to procedures, report incidents, and ensure proper documentation.

5.2 Haematology Supervisor:

- Oversee test performance, maintenance compliance, IQC and EQC

5.3 Quality Manager/Officer:

- Monitor compliance, maintain documentation, and follow corrective actions and EQC.

5.4 Hematopathologist/ pathologist:

- Oversight the validation, quality control and reporting the results.
- Guide laboratory staff in troubleshooting technical issues, QC, reporting with the HPLC analyzer and provide the corrective action for these issues.

6. Documentation and Reporting

Version	Description	Review Date
01	Initial release	August 2029

7. References:

- Bio-Rad Variant II HPLC Analyzer Operation Manual
- Bain BJ, Daniel Y, Henthorn J, de la Salle B, Hogan A, Roy NBA, Mooney C, Langabeer L, Rees DC; BSH Committee. Significant haemoglobinopathies: A guideline for screening and diagnosis: A British Society for Haematology Guideline: A British Society for Haematology Guideline. Br J Haematol. 2023 Jun;201(6):1047-1065. doi: 10.1111/bjh.18794. Epub 2023 Apr 19. PMID: 37271570.

8. Appendices:

8.1 Appendix1: REAGENT PREPARATION:

- **HbA2/F calibrator**
  1. Add 10.0 mls of Hb A2 / F diluent to the Hb A2 / F calibrator
  2. Swirl gently and mix to ensure complete dissolution
  3. Allow to stand for 10 minutes at room temperature
  4. Write the date and time on the new calibrator bottle. This is stable for about 10 days at 2-8°C after preparation.
  5. Prepare per day of analysis prior to sample run

- **whole blood primer**:
  1. Add 1.0 ml of distilled water
  2. Swirl gently to ensure complete mixing
  3. Allow to stand for 10 minutes at room temperature
  4. Record the time and date on the bottle. This reagent is stable for up to 21 days at 2-8°C.
  5. Prepare per day of analysis prior to sample run

- **HbA2/F Bi-level control (level I & II)**
  1. Reconstitute with 0.5 ml or 1.0ml of distilled water depend on the product sheet and refer to the product insert sheet for further instructions
  2. Dilute control HbA2 level 1 and 2 as patient sample (5µl into 1 ml wash/diluent solution)
  3. Prepare per day of analysis prior to sample run

8.2 Appendix 2:

Instrument Name & Model	**BIO-RAD VARIAN II**	**Serial No #**		**Manufacture**	
Commissioned by & Date		**Service Agency**		**Biomed No.**	
Engineer Contact No #		**Frequency of Calibration**		**Location**	

Month of						**Monthly maintenance done on**											**Periodic Maintenance Done on:**															
Date	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**
Daily maintenance																															
Check Buffer & wash level																															
Check Waste container																															
Pressure check Pump A (0) (20-60)±5																															
Pressure check Pump B (100) (20-60)±5																															
Cartridge Injection Count (<250)																															
Paper Supply																															
Leaks																															
Flash Piston Seal (30 ml)																															
Remove sample																															
Wipe spills																															
Cartridge Temp																															

HbA2/F control run (L1 & L2)																														
Initials																														
Comment																														

Monthly Maintenance	**Clean Barcod Reader**	**Clean Dilution Chamber**	**Clean Sample Needle**	**Clean & Inspect sample Rack**	**Decontaminate Sampling Fluid Path**	**Flush Piston and Seal Value**	**Clean Ext / Interior Face/ Belts**	**Comment initials**
Periodic Maintenance	**Backup Replace Database**	**Change Printer Toner**	**Change Sample Needle**	**Comment initials**	**Major break down during the month or PPM**			