National Standard Operating Procedure for Hemoglobin Electrophoresis

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Ministry of Health

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Document author	Dr. Musleh Al Musalhi
Designation	Senior Specialist Hematopathologist
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Validated by		**Approved by**	
Name	**Dr. Qamra Al-Sariri**	**Name**	**Dr. Badriya Al Rashidi**
Designation	**Director General of Quality Assurance Center**	**Designation**	**Director General of Directorate General of Health Services and Programs**
Signature		**Signature**	
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Table of Contents

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The taskforce is comprised of:

- Dr Sabria Nasser Al Hashami (Chairperson), head of haematology laboratory; Royal Hospital
- Dr Bushra Mahmmod Al Abri, head of laboratory; Al Nahdha Hospital
- Dr Musleh Mohammed Al Musalhi, Head of laboratory; Ibra Hospital
- Dr Hajer Mohammed Al Ghaithi, Head of Haematology Laboratory; Sohar Hospital
- Dr Yusra Hammad Al Abri, Haematopathologist; Nizwa Hospital
- Mr Hussain Ali Al Lawaty, Chief of scientists; Department of blood banks services
- Mrs Sumaya Ahmed Al Balushi (Member Secretary); Directorate general of health services and programs

Acronyms

SOP	Standard operative procedure
MOH	Ministry of Health
DGHS	Director General of Health Services
HPLC	High Performance Liquid Chromatography
IQC	Internal Quality Control
QC	Quality Control
P & P	Policy and Procedure
Hb	Hemoglobin
LIS	Laboratory information system
A2	Hemoglobin A2
EDTA	Ethylenediaminetetraacetic Acid
HbA	Adult Hemoglobin
HbC	Hemoglobin C
HbD	Hemoglobin D
HbE	Hemoglobin E
HbS	Sickle Hemoglobin
AFSC	Control (A, F, S, C)

1. **Purpose**

   This Test is performed as a second confirmation method for Haemoglobinopathy when abnormal Haemglobin variant detected i.e. HbC, HbD, HbE, Hb Lepore and unknown variants which have been tested by BIO-RAD Variant II Beta thalassaemia analyzer.

2. **Scope**

   This SOP is intended for laboratory technologists and hematopathologists to use and interpret hemoglobin electrophoresis.

3. **Definitions**

   - **Electrophoresis:** The migration of charged particles (ions) in a liquid or semisolid medium under the influence of an external electric field.
   - **Anode:** The positive electrode. In an electrolytic cell (like an electrophoresis tank), anions (negatively charged molecules) migrate toward the anode.
   - **Cathode:** The negative electrode. Cations (positively charged molecules) migrate toward the cathode.
   - **Hemolysate:** A solution prepared by lysing red blood cells with a hypotonic solution or detergent (e.g., Triton X-100), releasing hemoglobin into solution for analysis.
   - **Co-migration:** The phenomenon where two or more distinct protein variants exhibit identical electrophoretic mobility under a specific set of conditions, appearing as a single band.
   - **Homozygous:** Having two identical alleles for a specific globin gene (e.g., results in Hb SS phenotype).
   - **Heterozygous:** Having two different alleles (e.g., results in Hb AS phenotype).
   - **Compound Heterozygous:** Having two different mutant alleles at the same locus (e.g., results in Hb SC disease).

4. **Procedure**

   4.1.Clinical background:

   In physiologically normal adults, the principal hemoglobin constituent within erythrocytes is hemoglobin A (HbA), accompanied by minor fractions of hemoglobin A2 (HbA2) and fetal hemoglobin (HbF). Each hemoglobin molecule comprises two alpha globin chains and two non-alpha globin chains. In adult hemoglobin (HbA >95%), the non-alpha chains are classified as beta chains, whereas in fetal hemoglobin

(HbF <2% in adult), these are gamma chains. Hemoglobin A2 (HbA2 2.4-3.4%), a less abundant variant, consists of alpha and delta chains.

Acid and alkaline hemoglobin electrophoresis is implemented to achieve the precise differentiation and separation of minor hemoglobin variants, such as G and D, as well as E and O from S and C. This analytical methodology is employed for the confirmatory identification of hemoglobin variants previously detected via high-performance liquid chromatography (HPLC).

4.2.Principle:

Hemoglobin electrophoresis is an analytical technique based on the migration of hemoglobin molecules in an electric field, exploiting differences in their net charge at a specific pH. Under controlled conditions, hemoglobin variants—due to structural differences in their globin chains—exhibit distinct electrophoretic mobilities. When subjected to an electric current on a support medium such as cellulose acetate or agarose gel, these hemoglobin molecules separate into discrete bands according to difference in net electrical charge at a defined pH. This separation enables the identification and characterization of normal and abnormal hemoglobin fractions, facilitating the diagnosis of hemoglobinopathies and thalassemias. The method is highly sensitive in distinguishing between hemoglobin types that may co-migrate in other analytical systems, and is often performed in conjunction with alkaline and acid pH buffers to maximize diagnostic accuracy.

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 2 ml. Storage up to 7 days at 2-8°C. Sample can be saline washed and stored frozen at -80C for up to 6 months. Haemolysate are stable for up to 36 hours when stored at 2 – 8 °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 hemolysis

4.3.2 Reagents:

- Hb Gels

- Acid Violet Stain Concentrate
- Haemoglobin Lysing Agent
- Fixative solution
- Normal saline (0.85% NaCl)
- Distilled water
- Sample Applicator Blades
- Disposable sample cups
- REP prep. Solution
- Blotters

4.3.3 Materials

- Electrophoresis system
- Electrophoresis Staining system
- Sample applicator

4.3.4 Quality Control:

4.3.4.1 AFSC Hemo Control.

4.3.4.2 Bio-Rad Hb A2/F Controls (Level 1 & 2): Used to verify the lysing and general separation quality.

4.3.4.3 In-House Controls: Aliquots of known Hb SC or Hb AC patient samples are stored frozen (-20°C) in the freezer. These are used to supplement commercial controls, especially when troubleshooting resolution issues. In-house controlled should be validated and documented.

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

4.3.5 Safety precaution:

4.3.5.1 All specimens need to be treated as potentially infectious.

4.3.5.2 Standard procedures for handling biohazard material must be always followed.

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

4.4.Analytical stage:

4.4.1 Sample Preparation:

4.4.1.1 Labeling: Label a set of test tubes with the corresponding laboratory accession numbers.

4.4.1.2 Aliquot: Pipette 200 µL of well-mixed whole blood into the tube.

4.4.1.3 Saline Addition: Add approximately 1000 µL of 0.85% Saline.

4.4.1.4 Centrifugation: Centrifuge at 3500 rpm for 5 minutes.

4.4.1.5 Decanting: Carefully aspirate or decant the supernatant (plasma + saline). Be careful not to disturb the cell pellet.

4.4.1.6 Repeat: Repeat steps 3-5 two more times. Triple saline wash reduce plasma protein interference.

4.4.1.7 Final Pellet: After the last wash, remove as many supernatants as possible to leave a "dry" button of packed red cells.

4.4.2 Lysate Preparation: (target Hb 2-3 g/dl)

4.4.2.1 Standard Dilution:

- For samples with normal adult hemoglobin levels (12-15 g/dL):
- Mix 1 part Packed Cells (50 µL) + 4 parts Lysing Agent (200 µL).
- This is a 1:5 dilution.

4.4.2.2 Adjusted Dilution (Anemic Samples):

- For samples with low Hb, reduce the volume of Lysing Agent.
- Aim for a final hemoglobin concentration of 2.0 - 3.0 g/dL in the lysate.

4.4.2.3 Control Preparation:

- AFSC Control: Reconstitute with distilled water as per vial instructions.
- Optimization: If the commercial control gives faint bands, create a "Cocktail Control" : Mix 50 µL AFSC Control + 10 µL Hb SC Patient Sample + 10 µL Hb F (Cord Blood) + 250 µL Lysing Agent.
- Incubation: Vortex vigorously and let stand for 5 minutes.

4.4.3 Instrument Setup:

- Power Up
- Program
- Gel Placement
- Electrode Application

- Sample Loading
- Start Run

4.4.4 Staining and Processing:

4.4.4.1 Transfer:

- Upon completion of electrophoresis
- Remove electrodes.
- Gel Block Removal: Use the Gel Block Remover tool to slice off the thick agarose blocks at both ends of the gel. The blocks contain buffer salts that will contaminate the stain.
- Mounting: Attach the gel (now a flat sheet) to the frame. Ensure the gel side faces *outward*.

4.4.4.2 Staining Cycle:

- Load the frame into the stainer. Select Acid Hb/ alkaline Hb.
- Step 1: Fixation: The gel is immersed in Fixative. This precipitates the hemoglobin bands so they don't wash away.
- Step 2: Staining: Immersion in Acid Violet. The dye binds to the proteins.
- Step 3: Destain: Immersion in dilute Acetic Acid. This removes stain from the background agarose, leaving it clear, while the protein bands remain blue/violet.
- Step 4: Wash: A quick rinse with water to remove acid.
- Step 5: Drying: Hot air (65-70°C) typically for 10-15 mins dries the gel into a hard, transparent film. Note: Don’t exceed 70C to prevent gel cracking .

4.4.4.3 Finishing:

- Remove the gel. Label the back with the Date and Gel Number.

4.5.Post – analytical stage:

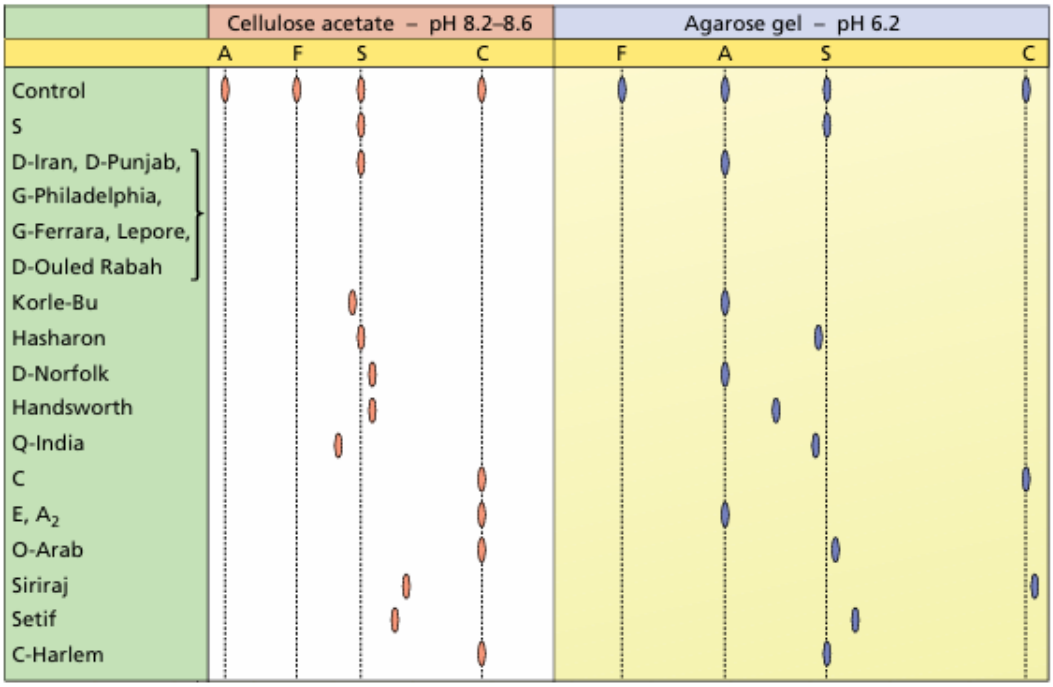
4.4.5 The possible identifying of the hemoglobin types present in the samples can be determined by visual evaluation of the completed gel compared to the controls which provide a marker for band identification. At the end of the run gel is validated along with the control and enters results to al Shifa system. Results are validated and released by HPLC Supervisor or trained experienced senior staff in the HPLC section. Interpretation must correlate with CBC indices and HPLC findings .

4.4.6 Interpretation Matrix:

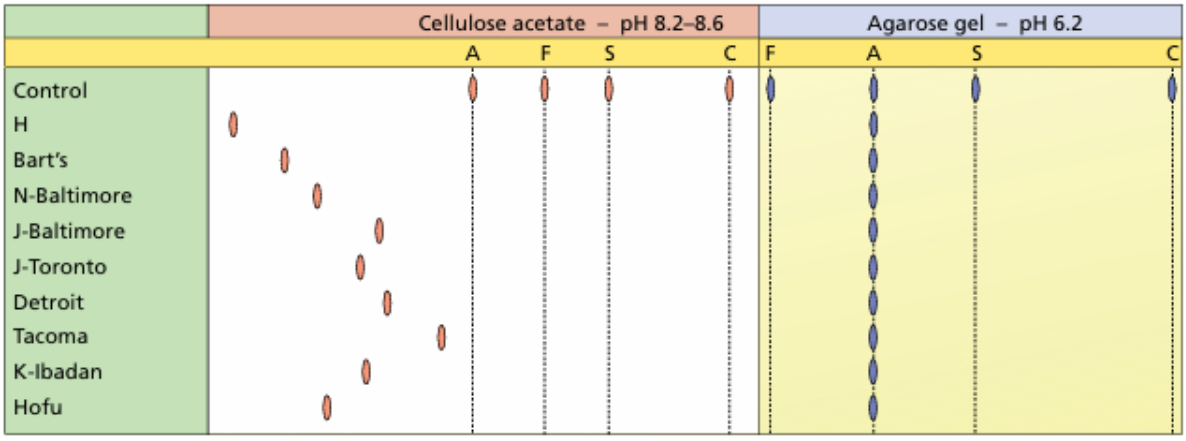
Condition	**Alkaline Gel (pH 8.6)**	**Acid Gel (pH 6.0)**	**Interpretation**
Normal	Band at A	Band at A	**Hb AA**
Sickle Trait	Bands at A, S	Bands at A, S	**Hb AS**
Sickle Disease	Band at S (no A)	Band at S (no A)	**Hb SS**
Hb D Trait	Bands at A, S	Single Band at A*	**Hb AD**
Hb C Trait	Bands at A, C	Bands at A, C	**Hb AC**
Hb E Trait	Bands at A, C	Single Band at A*	**Hb AE**
Hb SC Disease	Bands at S, C	Bands at S, C	**Hb SC**
Hb SE Disease	Bands at S, C	Bands at S, A	**Hb SE** (E moves to A)
Hb S-Oman	Bands A, Slow S	Bands A, and band between S/C	**Hb S-Oman**
Hb O-Arab	Band C	Near to S	**Hb O-Arab**

*Note: In Hb AD and Hb AE on Acid gel, the D and E variants co-migrate with A, so you see one large band at position A, appearing like a Normal AA, but the Alkaline gel/HPLC history tells you it is a variant.

Figure 1: Diagram showing the mobility of normal and variant haemoglobins on cellulose acetate at pH 8.2–8.6 in comparison with the mobility on agarose gel at pH 6.0–6.2: (a) haemoglobins with mobility close to A, S or C; (b) fast haemoglobins.



(a)



(b)

4.6 limitation of the test:

4.6.1Electrophoresis interpretation is unreliable in recently transfused patients (within 3 month)

4.6.2High hemoglobin F >20% interfere with separation of bands

4.6.3Neonatal sample

4.6.4Co-migration of hemoglobins at same position

4.6.5Cannot detect alpha thalassemia

4.6.6Cannot differentiate some rare variants

5. Responsibilities:

5.1 Laboratory Technologist:

- Operate instrument, perform maintenance, run and review QC.
- 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 analyzer and provide the corrective action for these issues.

6. Documentation and Reporting

Version	Description	Review Date
01	c	May 2031

7. References:

- **Helena Biosciences Europe.** (2015). *SAS-1 Acid Hb-12 Kit Instructions for Use*.
- **Helena Biosciences Europe.** *SAS-1 Plus Operator Manual*.
- **Helena Biosciences Europe.** *SAS-2 Auto Stainer Operator Manual*.
- **Royal Hospital Department of Laboratory Medicine.** *Technical Procedure: TP/HAEM/HPLC/04 - Acid Hb Electrophoresis*. Version 2.0.
- **Bain, B.J.** (2006). *Haemoglobinopathy Diagnosis*. 2nd Edition. Blackwell Publishing.
- **Dacie and Lewis Practical hematology , 12th edition**