



FETAL HEMOGLOBIN

Determination of fetal hemoglobin (HbF) in blood



ORDER INFORMATION

REF	Kit size
GD1020 00	30 determinations

INDICATION

Fetal hemoglobin (HbF) consists of two α chains and two β chains. It appears at the 3rd month of fetal life and reaches at birth 64-95% of total hemoglobin, it rapidly decreases towards the 6th month of life, reaching about 1% in the adult. Since an increase of HbF has been observed in many subjects suffering from Thalassemia Minor, the determination of this hemoglobin fraction serves as an additional datum in the diagnosis of the disease. HbF also increases in the drepanocytic anemia where it can reach 30% of the total hemoglobin.

PRINCIPLE

Hemoglobin F, unlike other physiological hemoglobins, is not denatured in a highly alkaline medium and then can be separated by filtration after selective precipitation of denatured hemoglobins. The remaining concentration of the HbF in the solution is photometrically determined and related to the total Hb.

COMPOSITION

REAGENT A:	1x45 ml
Sodium hydroxide	80 mmol/l
REAGENT B:	1x105 ml
Ammonium sulphate	2.8 mol/l
Hydrochloric acid	200 mmol/l
REAGENT C:	2x75 ml
Ammonium hydroxide	5 mol/l
REAGENT D:	1x50 ml
Toluol	
Flammable: F, xn, R11-20, S(2)16-25-29-33	
FILTER PAPER:	n. 30 sheets
Whatman 50	

Reagents preparation

The reagents are ready to use.

Storage and stability

Store at 15-25 °C. Do not freeze the reagents. The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

ANCILLARY EQUIPMENT

- Semi automatic pipettes of 100-1000 μ l
- Rocker or hematology rotator
- Chronometer
- Disposable test tubes
- Spectrophotometer or colorimeter set at 415 nm
- Analysis cuvettes (optical path = 1 cm)
- Centrifuge (2500-3000 rpm)
- Saline solution (NaCl, 9 g/l)

SPECIMEN

Whole blood with heparin or EDTA.

Stability: HbF is stable 7 days in blood stored at 2-8 °C or 3 months in the hemolysate frozen at -20 °C.

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

The reliability of test results should be monitored routinely using stable quality control materials and analyzed in the same manner employed for the unknowns.

PROCEDURE

Allow reagents to reach working temperature before using.

Hemolysate Preparation:

1. Wash erythrocytes 3 times with saline solution.
2. Hemolyze with 1.4 ml of distilled H₂O for each ml of erythrocytes.
3. Mix for at least 30 seconds.
4. Add 0.4 ml of Toluol (Reagent D) for each ml of erythrocytes.
5. Mix and centrifuge for 10 minutes at 2500-3000 rpm to separate the stromata from the hemolysate.
6. Discard the supernatant and filter hemolysate with filter paper.

Assay procedure:

Put in separated tubes:	HbF (1)	Hb Tot (2)
Hemolysate	0.1 ml	0.1 ml
Reagent A	1.5 ml	-
Exactly after 2 minutes of contact between alkaline solution and hemolysate, add:		
Reagent B	3.5 ml	-
Turn test tubes upside down for a few times and let settle for some minutes, then filter the content using selective filter paper.		
Reagent C	-	5 ml
Dilute the content of tubes (2) 1:20 with distilled water for the determination of total Hb. Determine absorbance of the solutions containing HbF and Hb Tot at 405-415 nm against a blank of Reagent C.		

CALCULATION OF RESULTS

Results should be determined as follows:

$$\% \text{ HbF} = \frac{A \text{ HbF}}{A \text{ Hb Tot}} \times 5$$

EXPECTED VALUES⁽¹⁾

HbF \leq 2%

Each laboratory should establish reference ranges for its own patient population.

ANALYTICAL PERFORMANCES

Precision

Precision has been tested on two samples: one at low HbF concentration from a blood donor, and a second one at high HbF concentration from umbilical funicle. The following results were obtained:

n=30	mean (%)	SD (%)	%CV
Sample 1	0.456	0.067	14.7
Sample 2	69.56	3.302	4.54

Linearity

Linearity has been tested on serial dilutions of a blood sample with high concentration of HbF (75%) with a sample with a low concentration (0.5%). The results obtained are the following:

$$y = 1.007x + 1.1699 \quad r = 0.999$$

Correlation

The present chemical method is correlable to the radial immunodiffusion one (R.I.D).

PRECAUTIONS IN USE

Reagent D is flammable: F, Xn; R11-20; S(2)16-25-29-33.

Refer to Safety Data Sheet.

The other reagents contain inactive components such as preservatives (Sodium Azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste management

Please refer to local legal requirements.

REFERENCES

1. HENRY J.B. Clinical Diagnosis and Management. 17th edition, Saunders Publisher (1984).
2. SINGER K., AMOZ M.V., CHERNOFF I. and LILY SINGER, Blood 413:29 (1951).
3. MARINGONI A., TORRELLI Quad. Sclavo Diagn. 21:117 (1985).
4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Approved Standard, 3rd Ed. (1999).
5. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.