

PRACTICAL HAEMOGLOBINOPATHIES

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Golden Rules to Evaluate Hemoglobin Electrophoresis

- 1) You must know the CBC results (RBC count, Hb, MCV, MCH, RDW & Plt).
- 2) Peripheral blood film might be useful (target, sickle, pencil, rhomboidal, golf).
- 3) Different methods have their own issues (gel: alkaline or acid, HPLC & capillary electrophoresis)
- 4) Family history and molecular tests are critical in difficult cases and to confirm the diagnosis.
- 5) As a physician, do not under estimate the medical history and clinical examination.

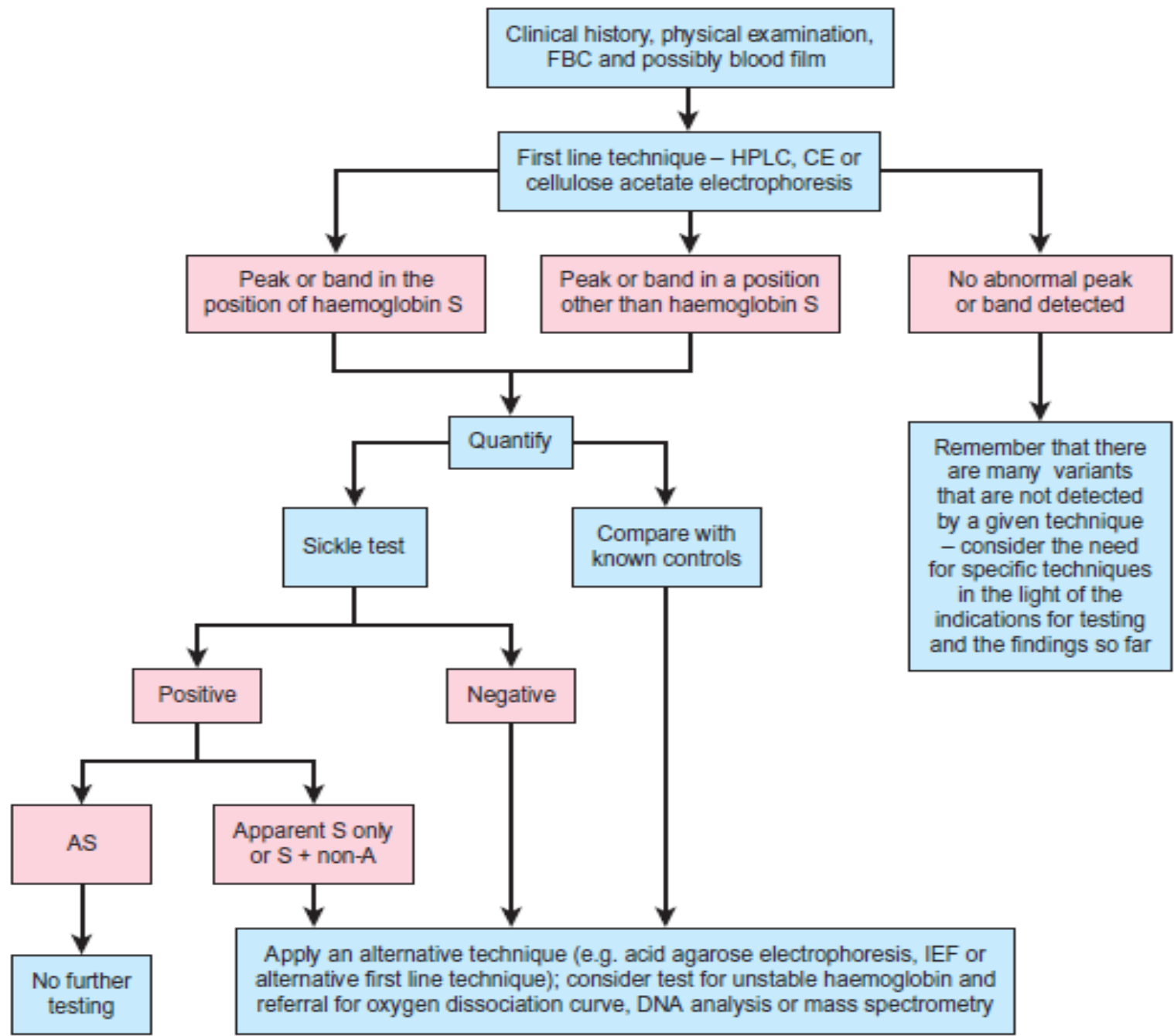




FIGURE 14-3 Schematic representation of relative mobilities of some abnormal haemoglobins. Cellulose acetate electrophoresis, pH 8.5.

Peak name	Calibrated area %	Area %	Retention time (min)	Peak area
P1	----	0.2	0.81	3314
F	23.8*	----	1.12	397 418
P2	----	3.1	1.33	53 378
P3	----	2.6	1.71	44 107
Ao	----	39.7	2.48	683 561
A2	1.8*	----	3.61	34 884
S-window	----	15.5	4.41	267 188
C-window	----	13.9	5.11	239 583

Total area: 1 723 434

F Concentration = 23.8%

A2 Concentration = 1.8%

*Values outside of expected ranges

Analysis comments:

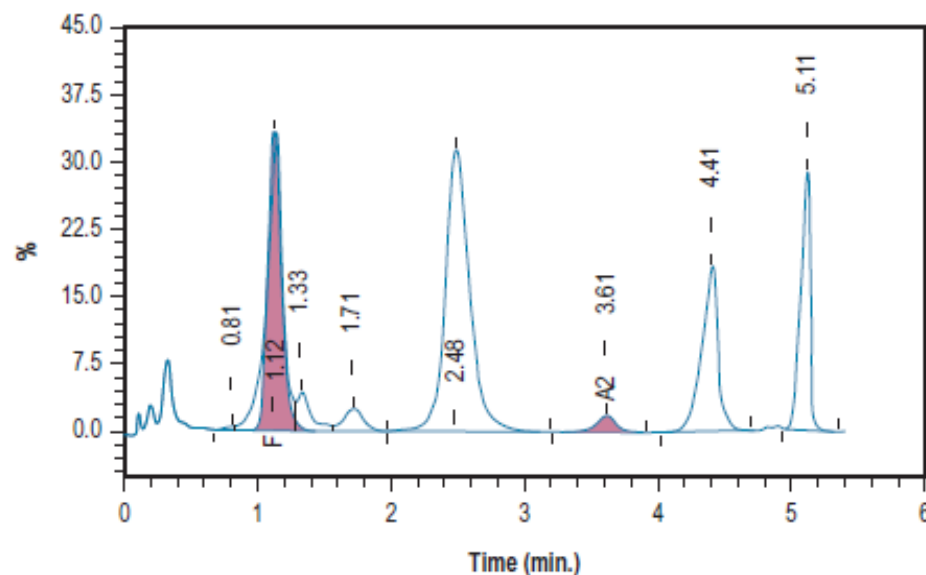


FIGURE 14-7 A mixture of haemoglobins separated by HPLC. From left to right the peaks are: injection artefact, post-translational modified haemoglobin F (double peak), haemoglobin F (pink), glycated haemoglobin A, other post-translationally modified haemoglobin A, haemoglobin A₀ (pink), haemoglobin S, post-translationally modified haemoglobin C (two very small peaks) and haemoglobin C.

Parameter	Iron deficiency anemia	α -thalassemia minor	β -thalassemia minor
MCV	↓	↓	↓
RDW	↑	Normal	Normal
RBCs	↓	Normal	Normal
Peripheral smear	Microcytosis, hypochromia Pencil cell	Target cells	Target cells
Serum iron studies	↓ Iron & ferritin ↑ TIBC	Normal/ ↑ iron & ferritin (RBC turnover)	Normal/ ↑ iron & ferritin (RBC turnover)
Response to iron supplementation	↑ Hemoglobin	No improvement	No improvement
Hemoglobin electrophoresis	Normal	Normal	↑ Hemoglobin A2

MCV = mean corpuscular volume; RBC = red blood cell; RDW = red cell distribution width; TIBC = total iron-binding capacity.

TABLE 14-5**RESULTS OF LABORATORY INVESTIGATIONS IN INTERACTIONS OF HAEMOGLOBIN S AND α OR β THALASSAEMIA IN ADULTS**

	MCV	% S	% A	% A₂	% F
AS	N	35–38	62–65	<3.5	<1
SS	N	88–93	0	<3.5	5–10
S/ β^0 thalassaemia	L	88–93	0	>3.5	5–10
S/ β^+ thalassaemia	L	50–93	3–30	>3.5	1–10
S/HPFH	N	65–80	0	<3.5	20–35
AS/ α^+ thalassaemia	N/L	28–35	62–70	<3.5	<1
AS/ α^0 thalassaemia	L	20–30	68–78	<3.5	<1
SS/ α thalassaemia	N/L	88–93	0	<3.5	1–10

HPFH, hereditary persistence of fetal haemoglobin; L, low; MCV, mean cell volume; N, normal.

Golden Rules to Evaluate Hemoglobin Electrophoresis

- 6) Are all normal hemoglobin variants present? And if present, are they in normal amount?
- 7) Beta thalassemia trait has a higher Hb A₂ (>3.6) & beta thalassemia major has a very high Hb F (>80%).
- 8) Is there any abnormal Hb? What is the percentage?
- 9) Sickle cell trait has 35% - 45% Hb S. If it is >45%, it is a sickle cell disease (when high Hb A₂ then likely S/beta thal).
- 10) Alpha thalassemia reduced other abnormal Hb level, in trait state.

KKUH

Heamatology Unit
Hb Electrophoresis

Hospital No.: QC Hb AFSC CONTROL-

ID : Hb AFSC CONTROL-2

Sample num.: 2
Z15

Date : 8/12/2009

Z14 Z13

Z12

Z11

Z10

Z9 Hb A

Z8

Z7

Z6

Z5

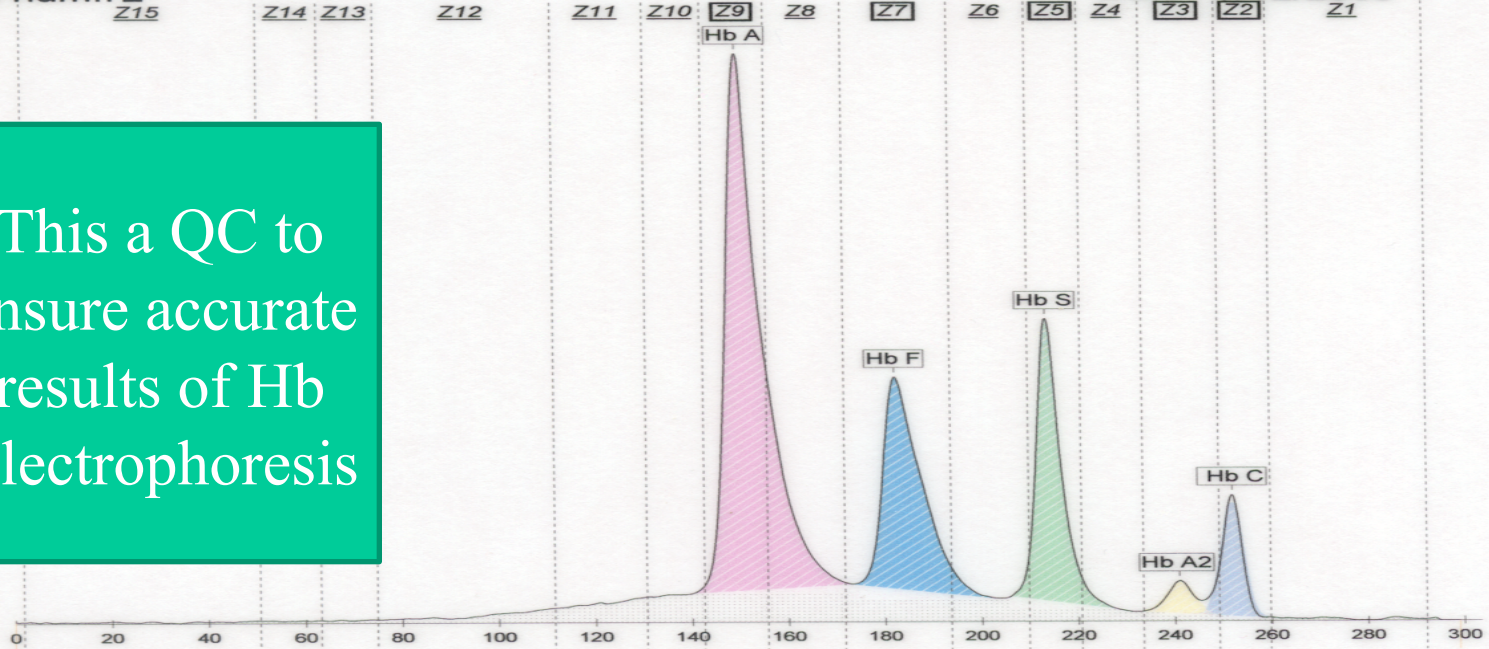
Z4

Z3

Z2

Z1

This a QC to
ensure accurate
results of Hb
Electrophoresis



Hb Electrophoresis

Fractions	%	Ref. %
Hb A	51.3	46.7 - 56.9
Hb F	21.4	17.4 - 22.4
Hb S	18.3	17.3 - 22.3
Hb A2	2.3	2.1 - 3.3
Hb C	6.7	4.6 - 7.0

KKUH

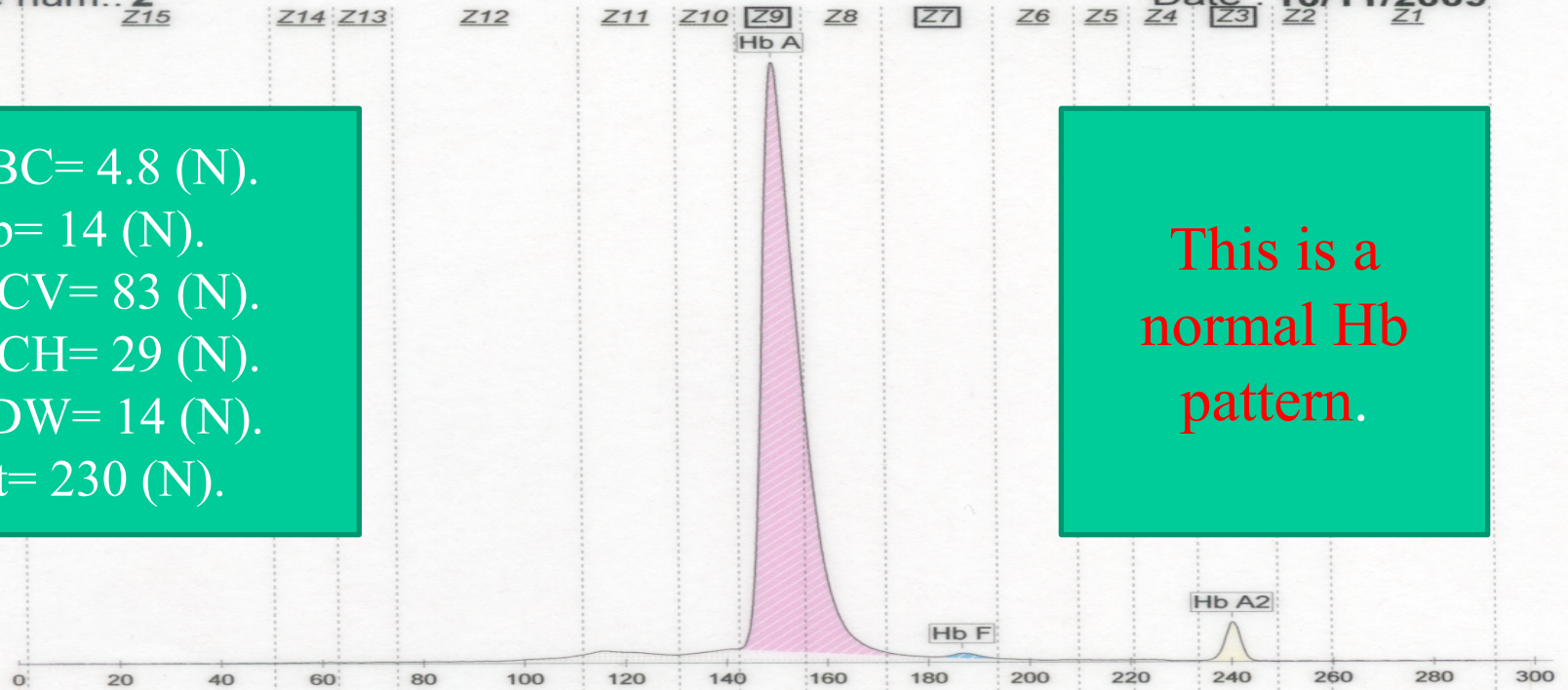
Heamatology Unit
Hb Electrophoresis

Hospital No.: 933376

ID : 061773

Sample num.: 2
Z15

Date: 10/11/2009



RBC= 4.8 (N).
Hb= 14 (N).
MCV= 83 (N).
MCH= 29 (N).
RDW= 14 (N).
Plt= 230 (N).

This is a
normal Hb
pattern.

Hb Electrophoresis

Fractions	%	Ref. %
Hb A	96.7	96.8 - 97.8
Hb F	0.5	=< 2.0
Hb A2	2.8	1.5 - 3.5

<

KKUH

Heamatology Unit
Hb Electrophoresis

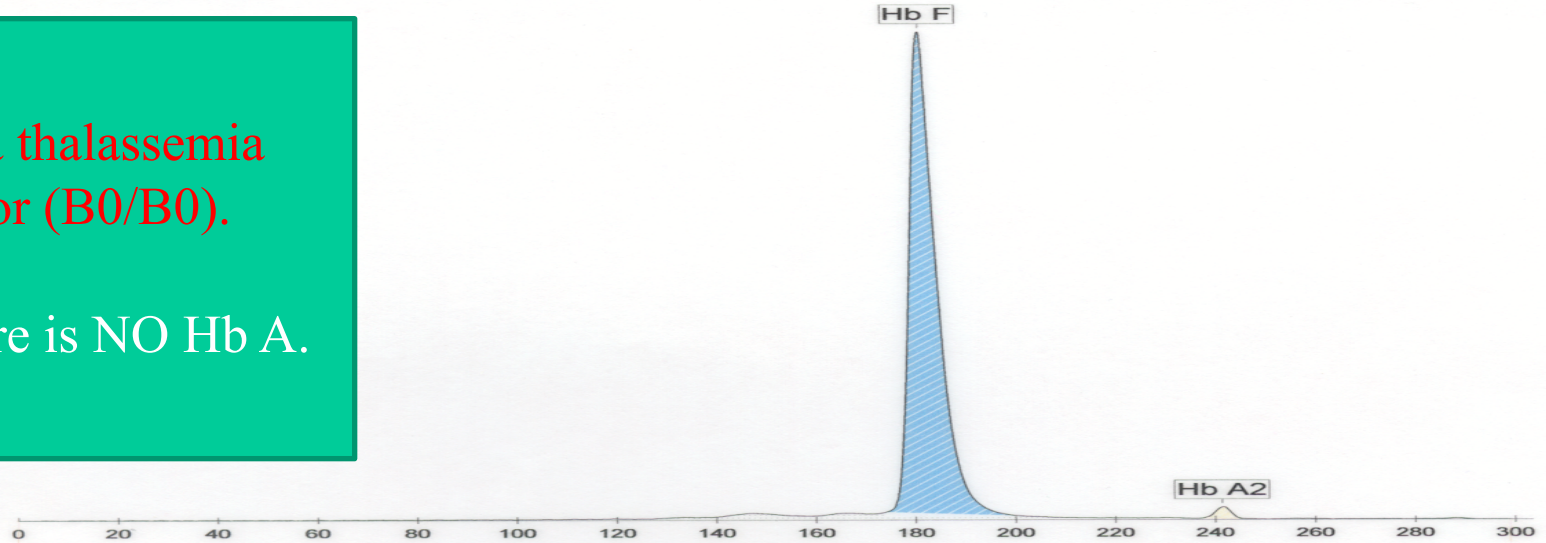
INSTRUMENT ID : KKHU : 24509

Hospital No.: 921107
Sample No 54

ID : 063761
Date : 09/05/2010

Beta thalassemia
major (B0/B0).

There is NO Hb A.



Fractions	%	Ref. %
Hb F	98.5	
Hb A2	1.5	

Comment :

28/3/2010
CBC Hb 98
MCV 73
NRBC 34

KKUH

Heamatology Unit
Hb Electrophoresis

INSTRUMENT ID : KKHU : 24509

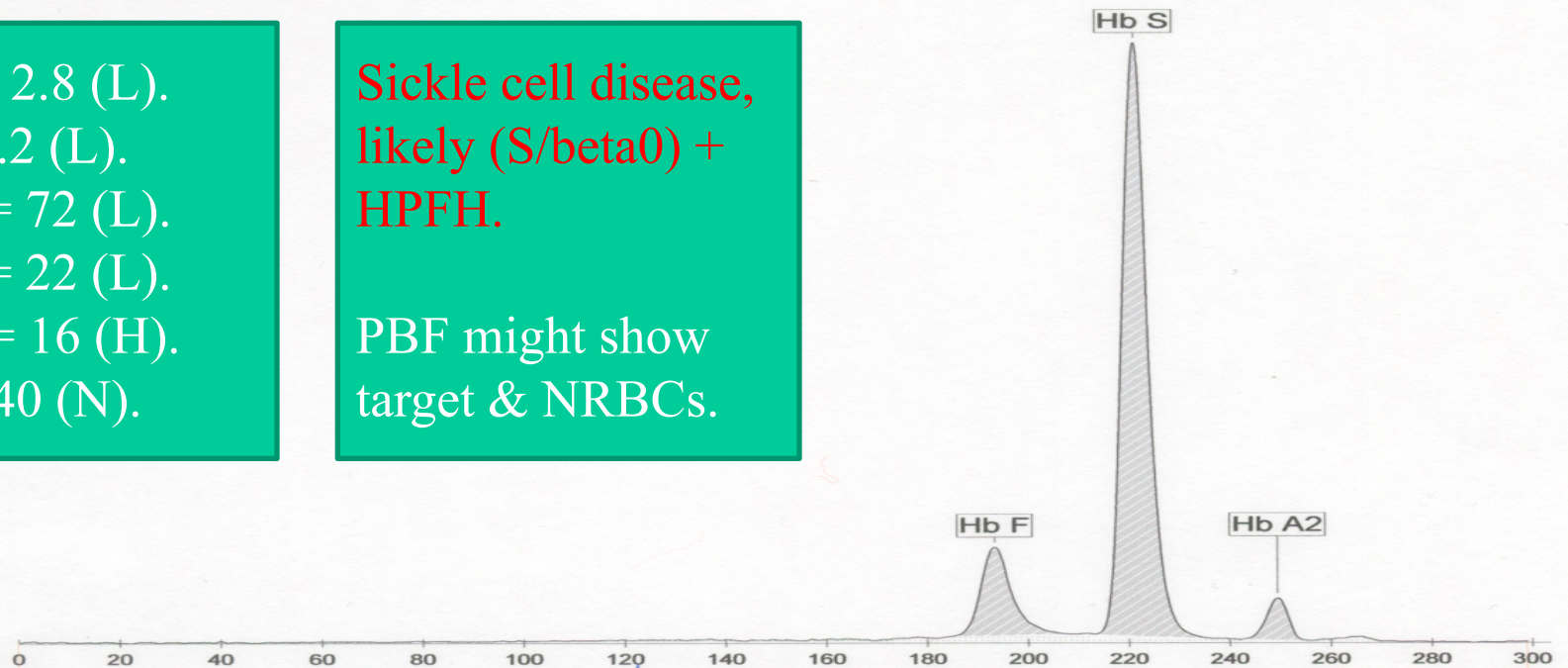
Hospital No.: 233095
Sample No 20

ID : 063478
Date : 17/04/2010

RBC= 2.8 (L).
Hb= 7.2 (L).
MCV= 72 (L).
MCH= 22 (L).
RDW= 16 (H).
Plt= 340 (N).

Sickle cell disease,
likely (S/beta0) +
HPFH.

PBF might show
target & NRBCs.



Fractions	%	Ref. %
Hb F	14.7	
Hb S	80.5	
Hb A2	4.8	

KKUH

Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKHU : 24509

Hospital No.: 594729

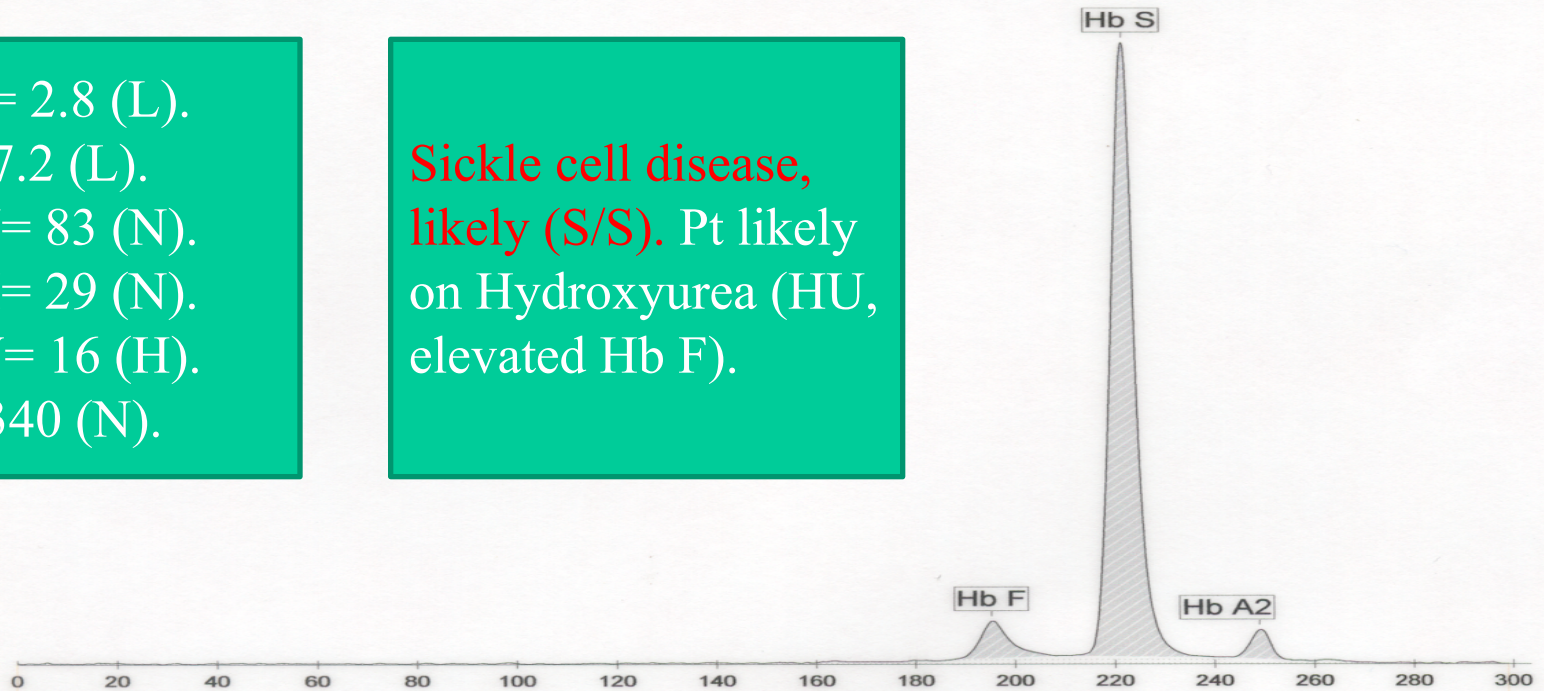
ID : 064199

Sample No 37

Date : 27/06/2010

RBC= 2.8 (L).
Hb= 7.2 (L).
MCV= 83 (N).
MCH= 29 (N).
RDW= 16 (H).
Plt= 340 (N).

Sickle cell disease,
likely (S/S). Pt likely
on Hydroxyurea (HU,
elevated Hb F).



Fractions	%	Ref. %
Hb F	6.5	
Hb S	89.9	
Hb A2	3.6	

KKUH

Heamatology Unit
Hb Electrophoresis

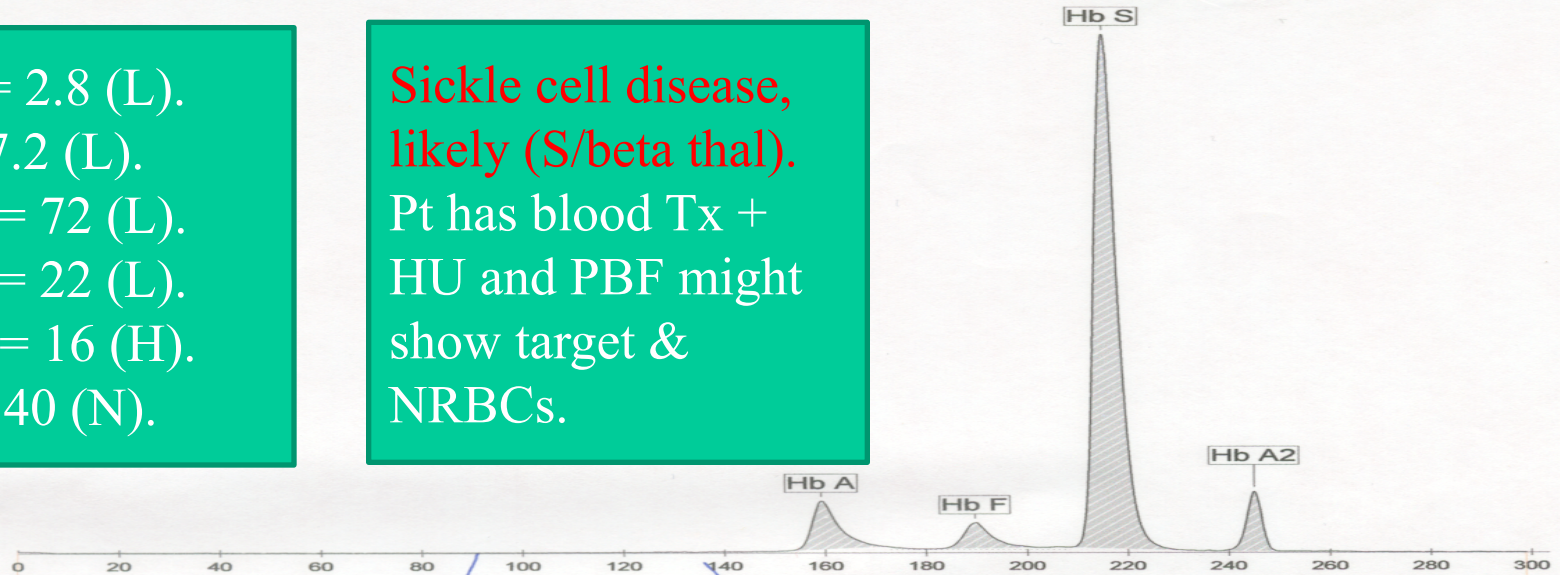
INSTRUMENT ID : KKHU : 24509

Hospital No.: 913628
Sample No 34

ID : 063511
Date : 19/04/2010

RBC= 2.8 (L).
Hb= 7.2 (L).
MCV= 72 (L).
MCH= 22 (L).
RDW= 16 (H).
Plt= 340 (N).

Sickle cell disease,
likely (S/beta thal).
Pt has blood Tx +
HU and PBF might
show target &
NRBCs.



Fractions	%	Ref. %
Hb A	8.7	
Hb F	4.9	
Hb S	80.1	
Hb A2	6.3	

Comment :

Homozygous sickle cell thal

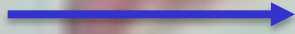
Many target cells & nucleated RBCs and sickle cell seen.

If Hb A2 is elevated with low MCV & MCH, this SCD likely (S/Beta thal) genotype.

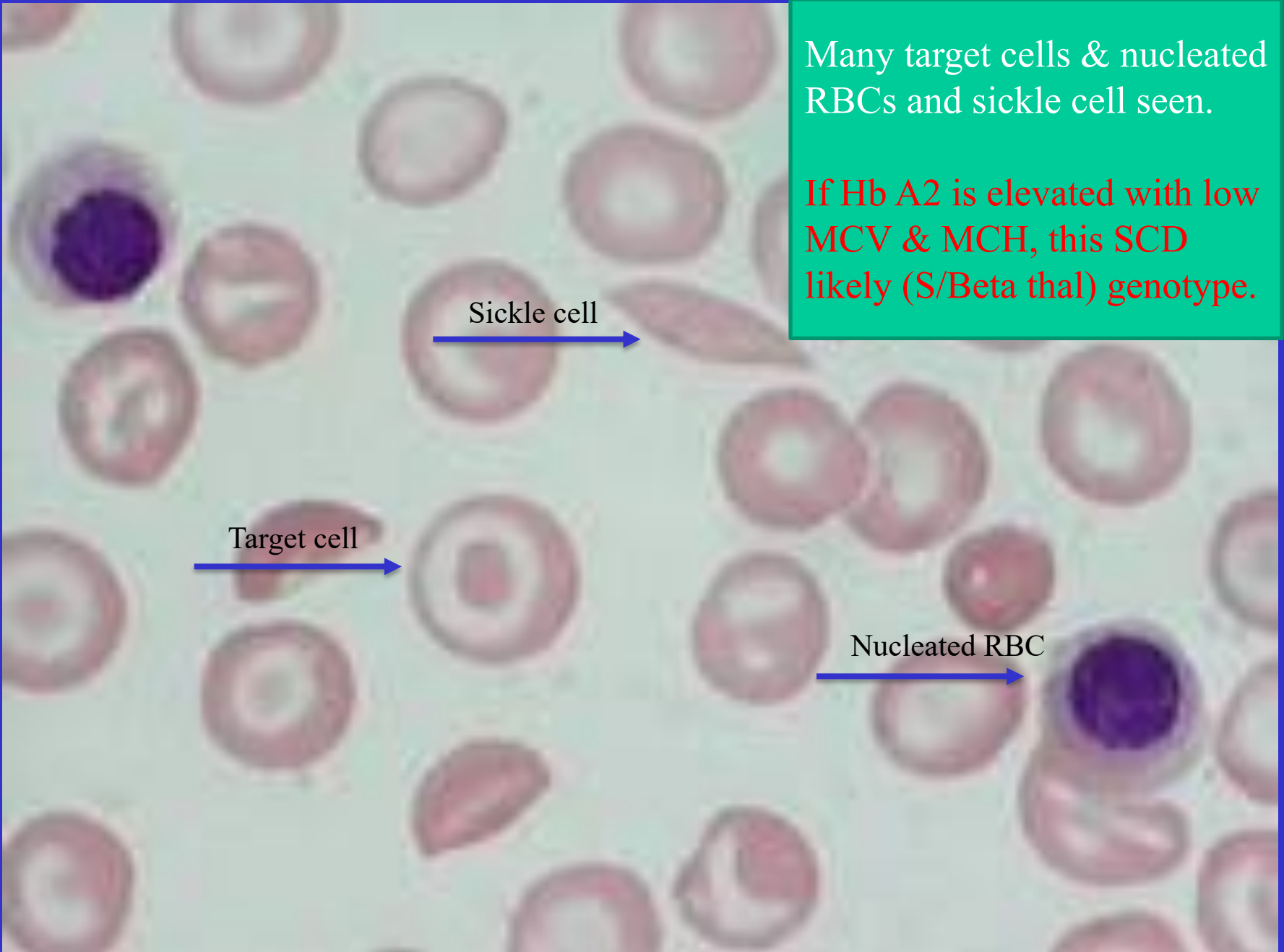
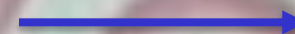
Sickle cell



Target cell



Nucleated RBC





KKUH

Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKHU : 24509

Hospital No.: 610043

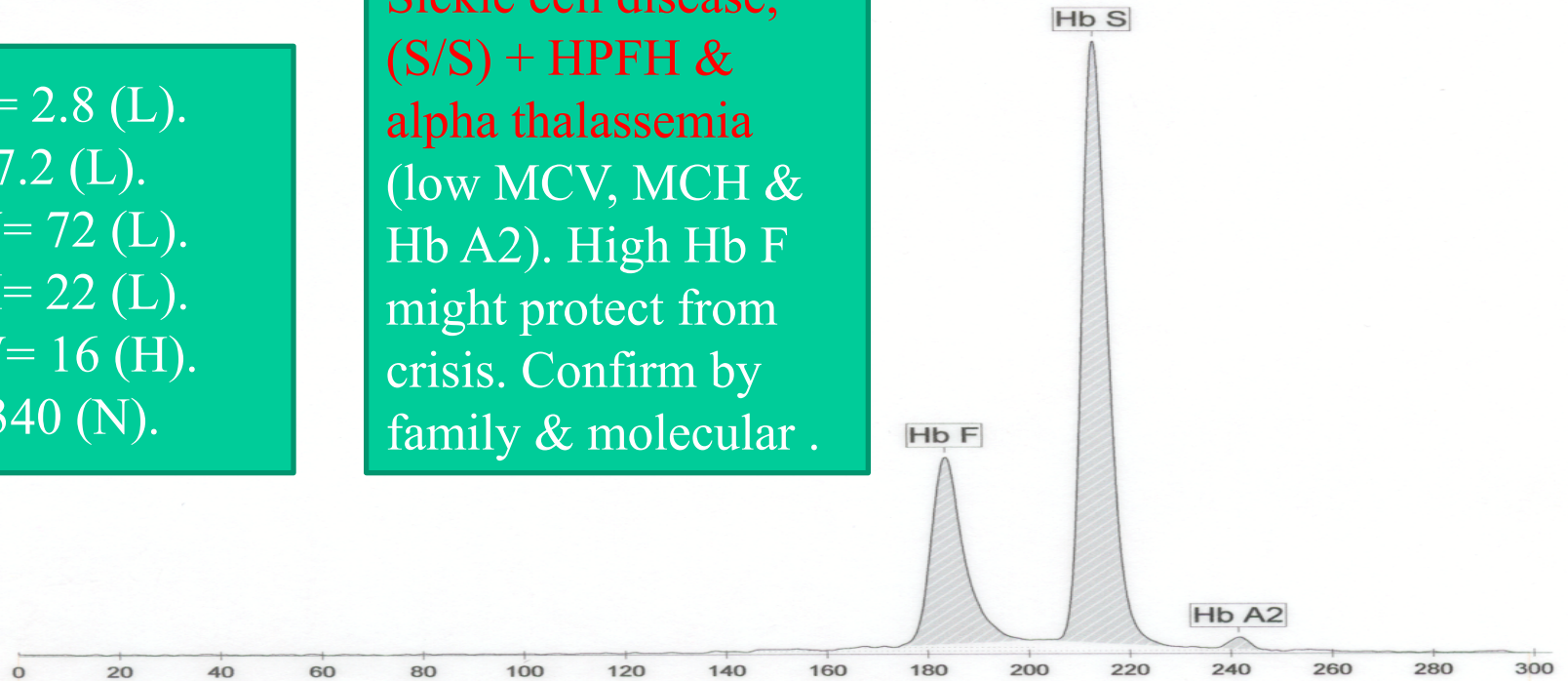
Sample No 52

ID : 064229

Date : 29/06/2010

RBC= 2.8 (L).
 Hb= 7.2 (L).
 MCV= 72 (L).
 MCH= 22 (L).
 RDW= 16 (H).
 Plt= 340 (N).

Sickle cell disease,
 (S/S) + HPFH &
 alpha thalassemia
 (low MCV, MCH &
 Hb A2). High Hb F
 might protect from
 crisis. Confirm by
 family & molecular .



Fractions	%	Ref. %
Hb F	28.1	
Hb S	70.8	
Hb A2	1.1	

KKUH

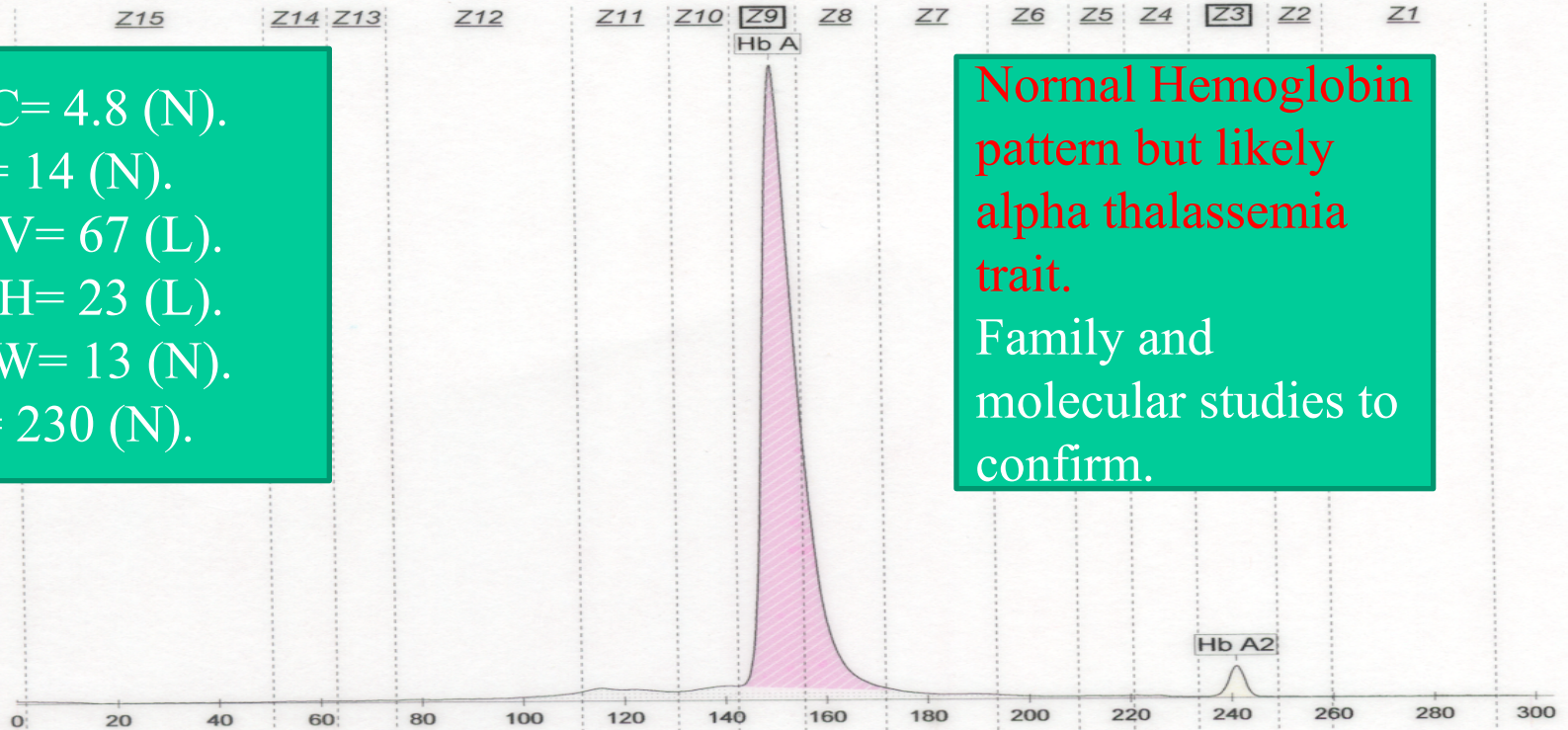
Heamatology Unit

Hb Electrophoresis

INSTRUMENT ID : KKHU : 24509

Hospital No.: Rack: SEBIA Pos.: 2
Sample No 20

ID : ABDULLAH
Date : 19/05/2010



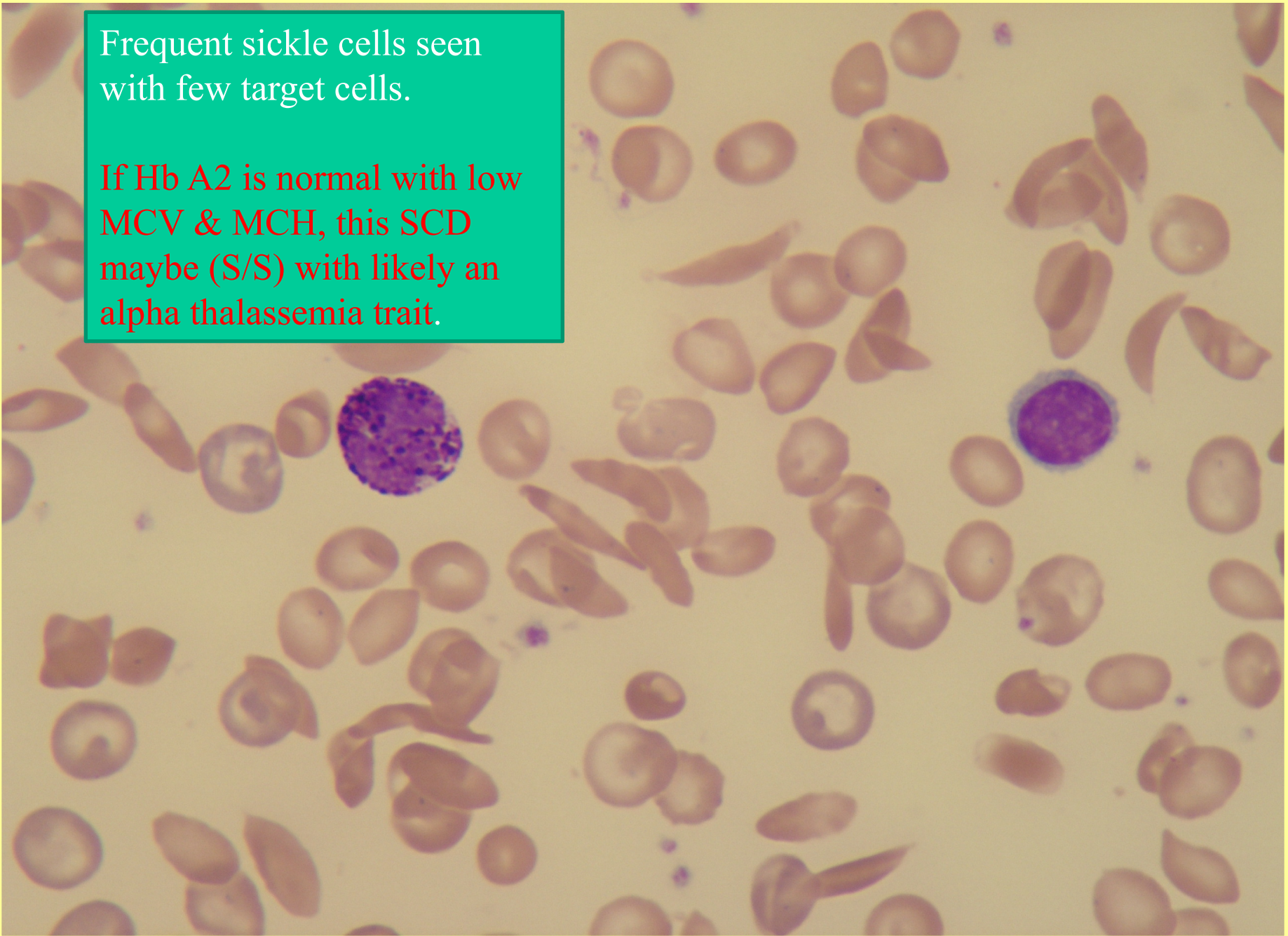
RBC= 4.8 (N).
Hb= 14 (N).
MCV= 67 (L).
MCH= 23 (L).
RDW= 13 (N).
Plt= 230 (N).

Normal Hemoglobin
pattern but likely
alpha thalassemia
trait.
Family and
molecular studies to
confirm.

Fractions	%	Ref. %
Hb A	97.7	95.0 - 99.0
Hb A2	2.3	1.5 - 3.5

Frequent sickle cells seen
with few target cells.

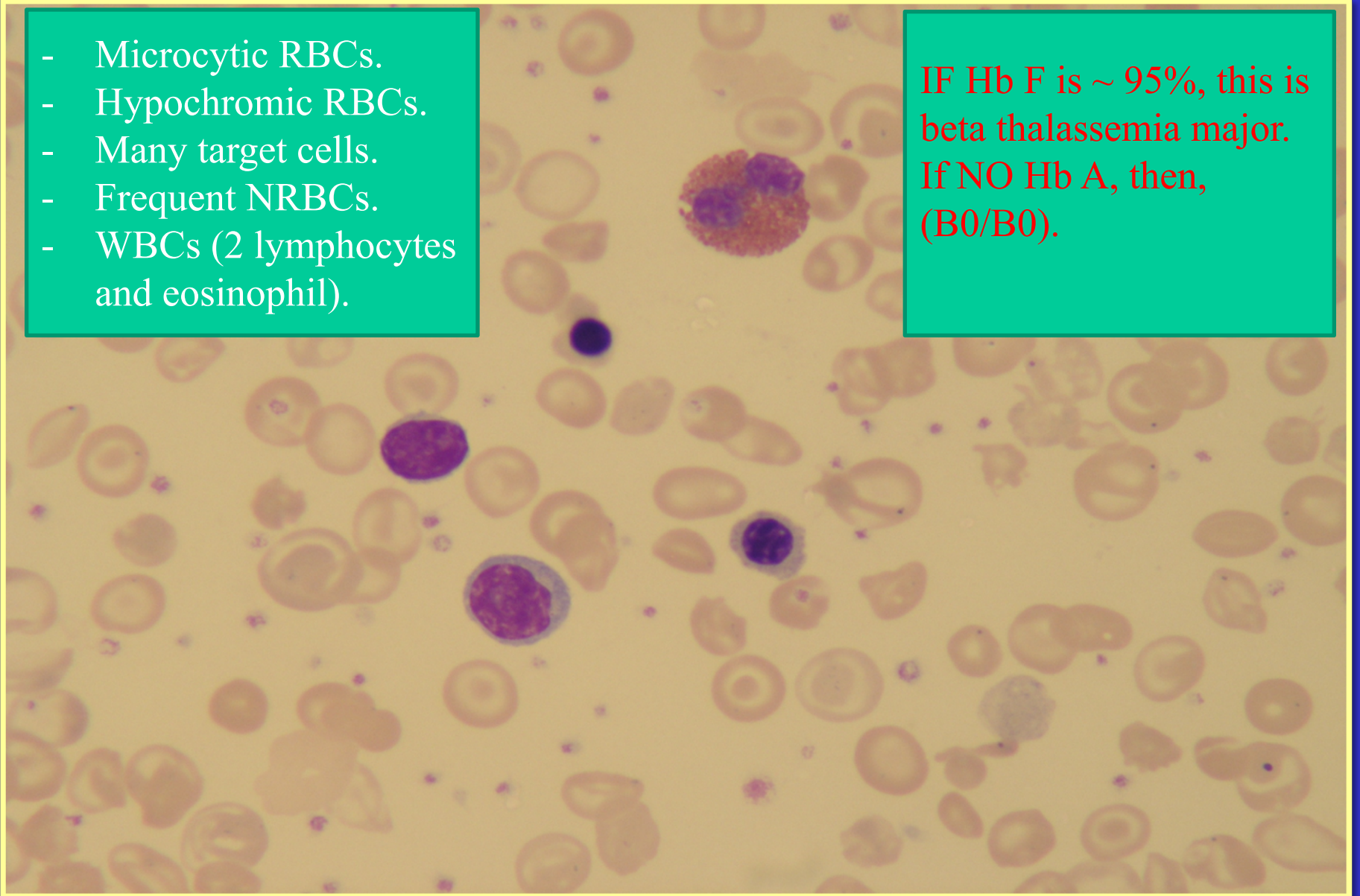
If Hb A2 is normal with low
MCV & MCH, this SCD
maybe (S/S) with likely an
alpha thalassemia trait.



Beta Thalassaemia Major

- Microcytic RBCs.
- Hypochromic RBCs.
- Many target cells.
- Frequent NRBCs.
- WBCs (2 lymphocytes and eosinophil).

IF Hb F is ~ 95%, this is beta thalassaemia major.
If NO Hb A, then,
(B0/B0).

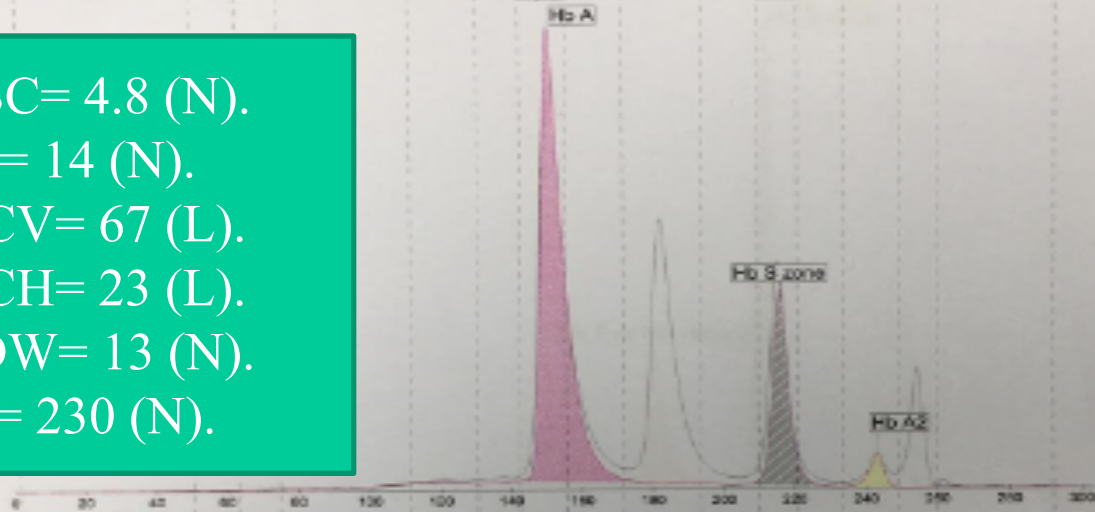


ACCESSION : 011832300169A

Date : 19/Nov/18

Z15 Z14 Z13 Z12 Z11 Z10 Z(A) Z8 Z(F) Z(D) Z(S) Z(E) Z(A2) Z(C) Z1

RBC= 4.8 (N).
 Hb= 14 (N).
 MCV= 67 (L).
 MCH= 23 (L).
 RDW= 13 (N).
 Plt= 230 (N).



Fractions	%	Ref. %
Hb A	73.9	
Hb S zone	22.6	
Hb A2	3.5	

Sickle cell trait
 with likely alpha
 thalassemia.

Family and
 molecular studies
 to confirm.

Comment : Solubility Test: Positive (+ve)

SOLUBILITY TEST

Signature

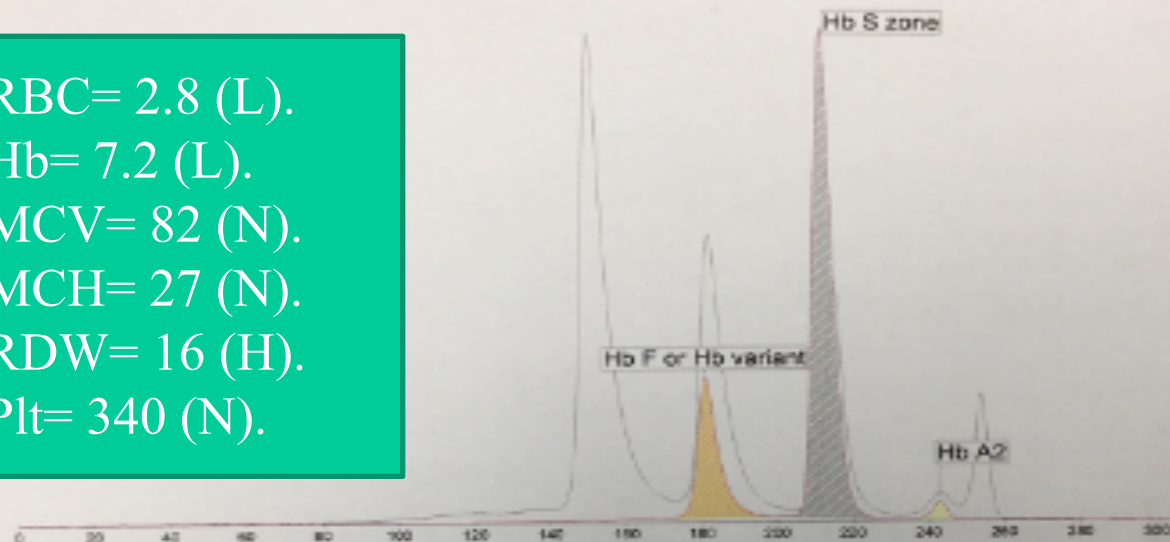
MRN.: 00-94-65-29

Depart. K MED Clinic

ACCESSION : 011832204545A

Date : 19/Nov/18

RBC= 2.8 (L).
Hb= 7.2 (L).
MCV= 82 (N).
MCH= 27 (N).
RDW= 16 (H).
Plt= 340 (N).



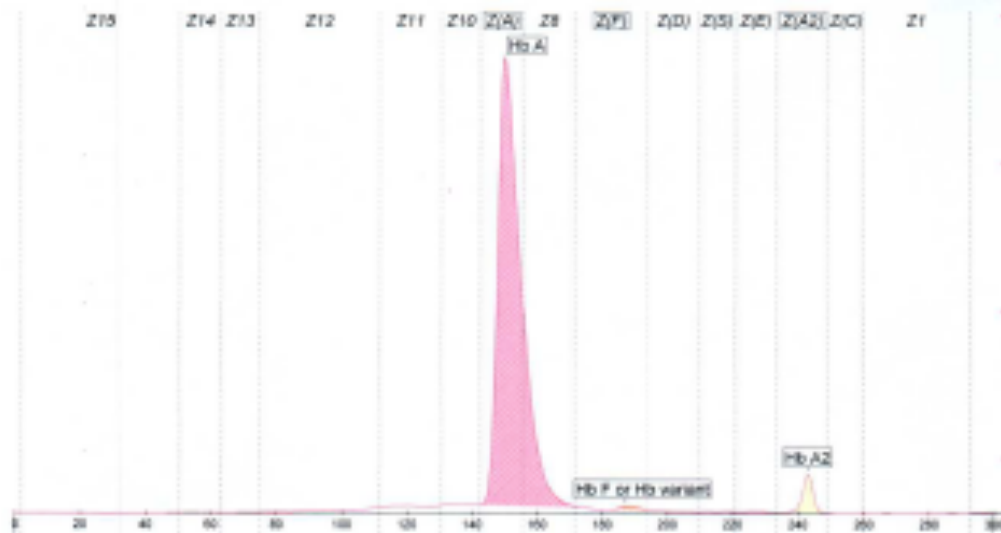
Fractions	%	Ref. %
Hb F or Hb variant	23.2	
Hb S zone	74.6	
Hb A2	2.2	

Comment : Solubility Test: Positive (+ve)

- Sickle cell disease, (S/S) + HPFH.
- (normal MCV, MCH & Hb A2). High Hb F might protect from crisis.
- Confirm by family & molecular .

Sample number:27

Date : 7/8/2018



Name	%	Normal Values %
Hb A	95.5<	96.8 - 97.8
Hb F or Hb variant	0.8 >	= < 0.5
Hb A2	3.7 >	2.2 - 3.2

Normal results even with slight elevated Hb A2 (NOT beta thalassemia trait). Family and molecular studies to confirm.

WBC: 3.1 ↓
RBC: 4.4
Hb: 12.5
MCV: 84.9
MCH: 28.4
RDW: 13.7
PH: 336

Comments

SOLUBILITY TEST

Signature



Thank you