PRACTICAL HAEMOGLOBINOPATHIES

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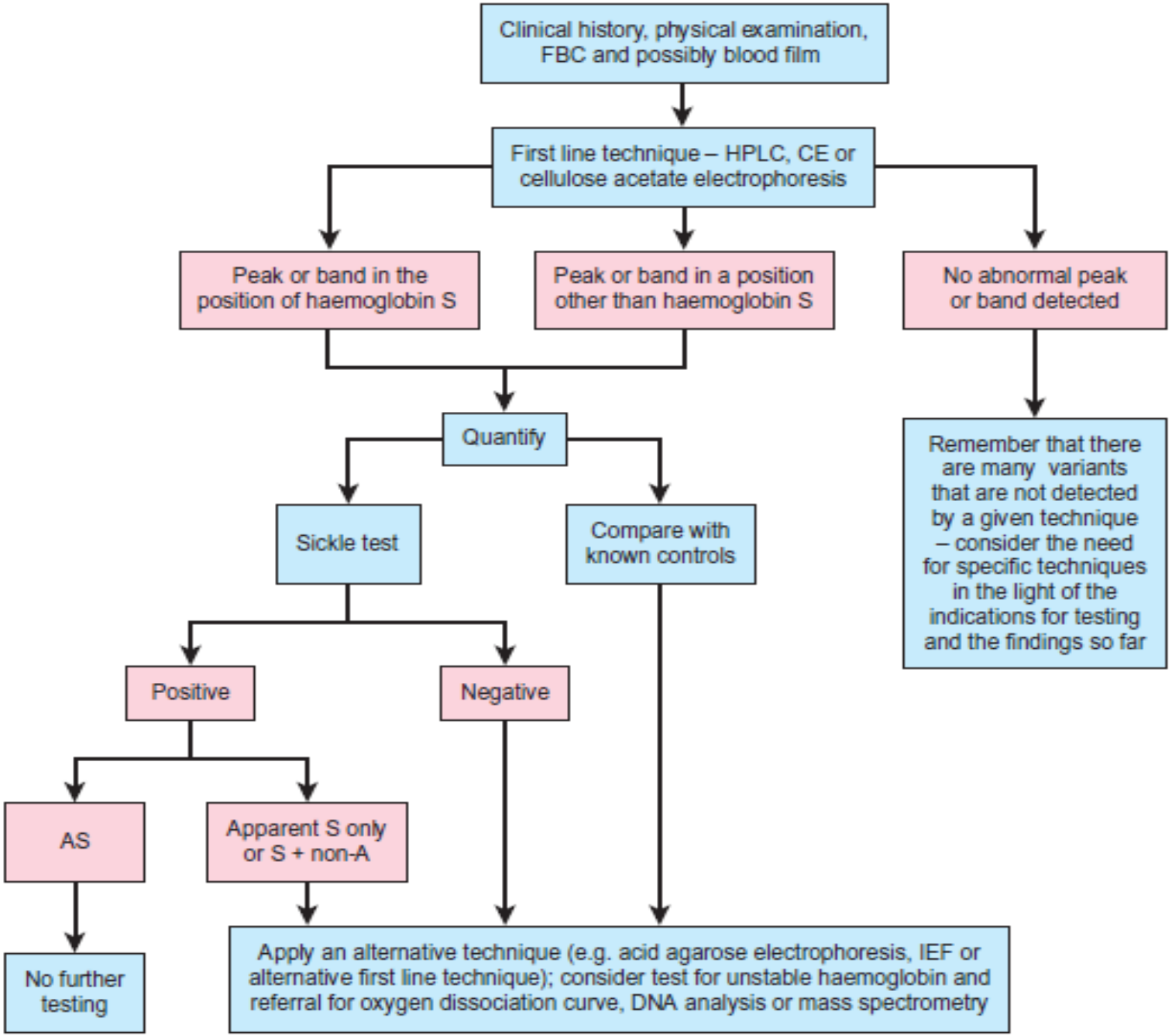
Golden Rules to Evaluate Hemoglobin Electrophoresis

- 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 has its 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.


Clinical history, physical examination, FBC and possibly blood film
First line technique – HPLC, CE or cellulose acetate electrophoresis
Peak or band in the position of haemoglobin S
Peak or band in a position other than haemoglobin S
No abnormal peak or band detected
Quantify
Sickle test
Compare with known controls
Remember that there are many variants that are not detected by a given technique – consider the need for specific techniques in the light of the indications for testing and the findings so far
Positive
Negative
AS
Apparent S only or S + non-A
No further testing
Apply an alternative technique (e.g. acid agarose electrophoresis, IEF or alternative first line technique); consider test for unstable haemoglobin and referral for oxygen dissociation curve, DNA analysis or mass spectrometry

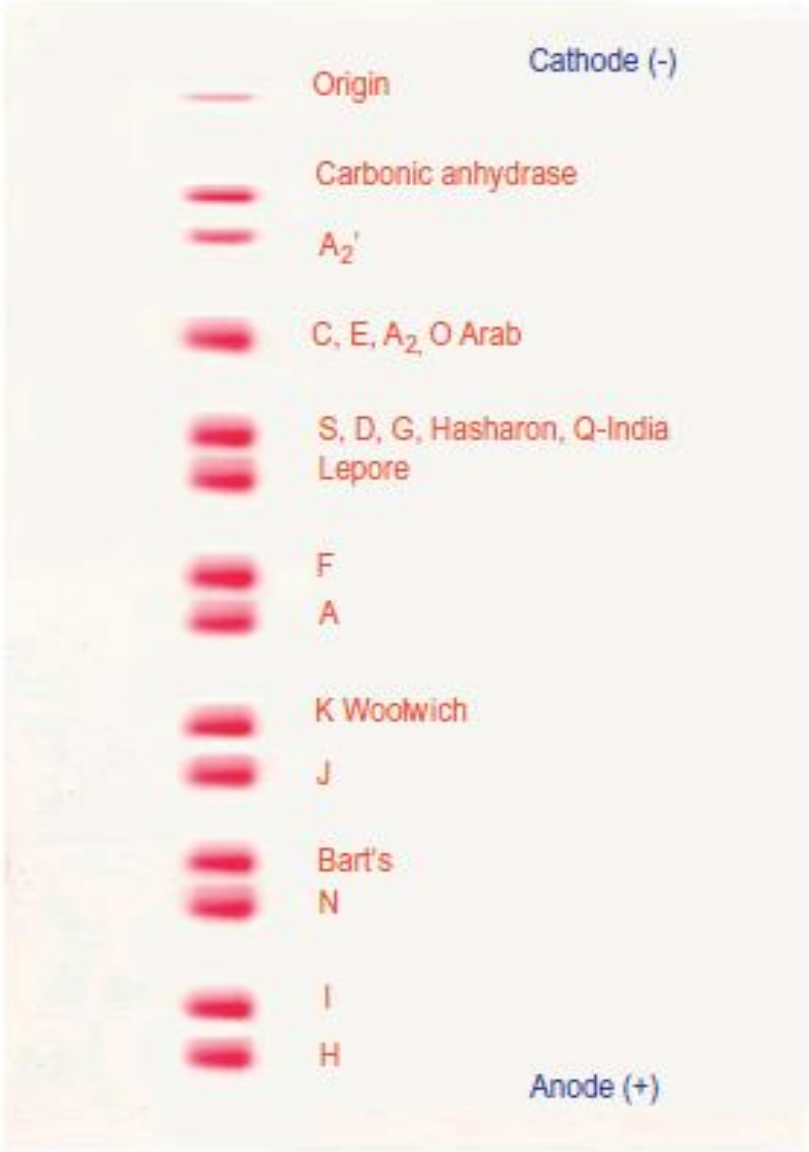


FIGURE 14-3 Schematic representation of relative mobilities of some abnormal haemoglobins. Cellulose acetate electrophoresis, pH 8.5.

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:

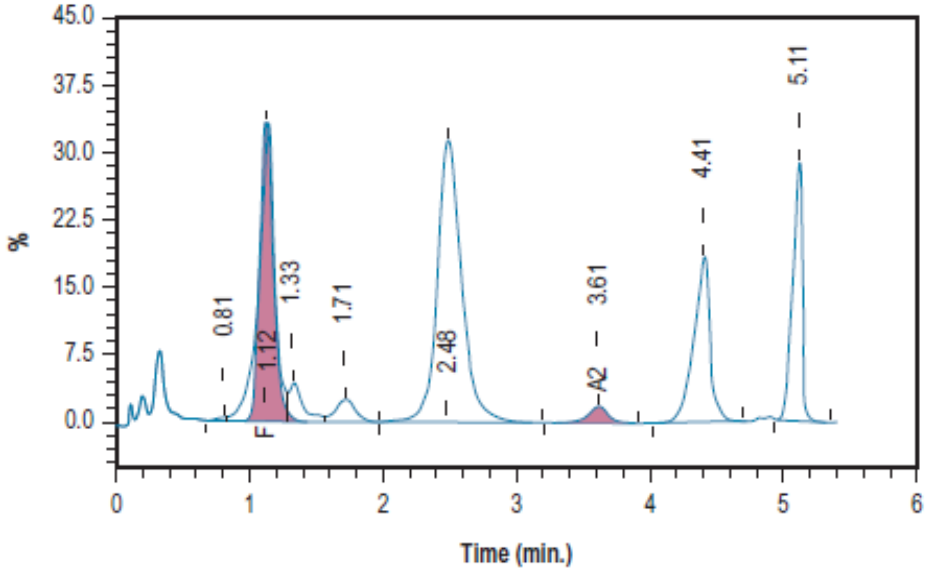


FIGURE 14-7 A mixture of haemoglobins separated by HPLC. From left to right the peaks are: injection artefact, post-translational modified haemoglobin F (double peak), haemoglobin F (pink), glycated haemoglobin A, other post-translationally modified haemoglobin A, haemoglobin A_0 (pink), haemoglobin 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	% A_2	% 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.

KKUH

Heamatology Unit

Hb Electrophoresis

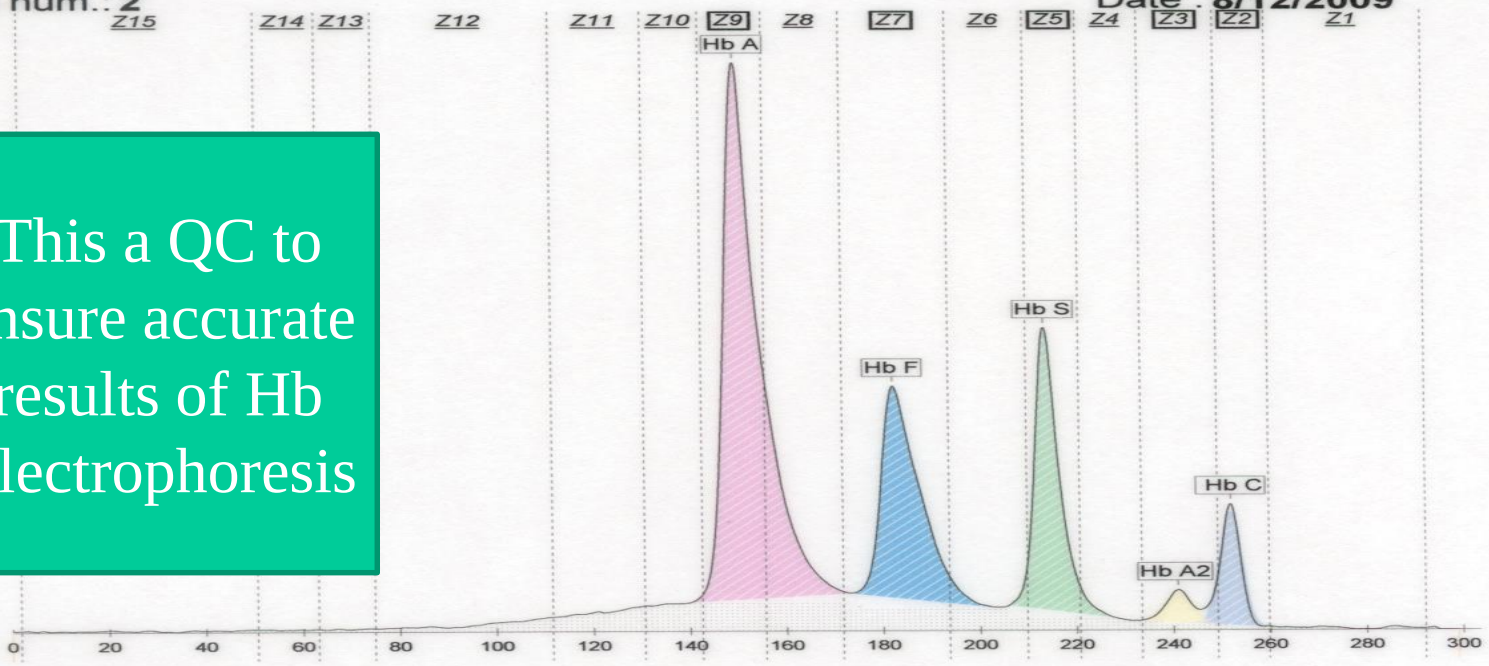
Hospital No.: QC Hb AFSC CONTROL-

ID : Hb AFSC CONTROL-2

Sample num.: 2

Date : 8/12/2009

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

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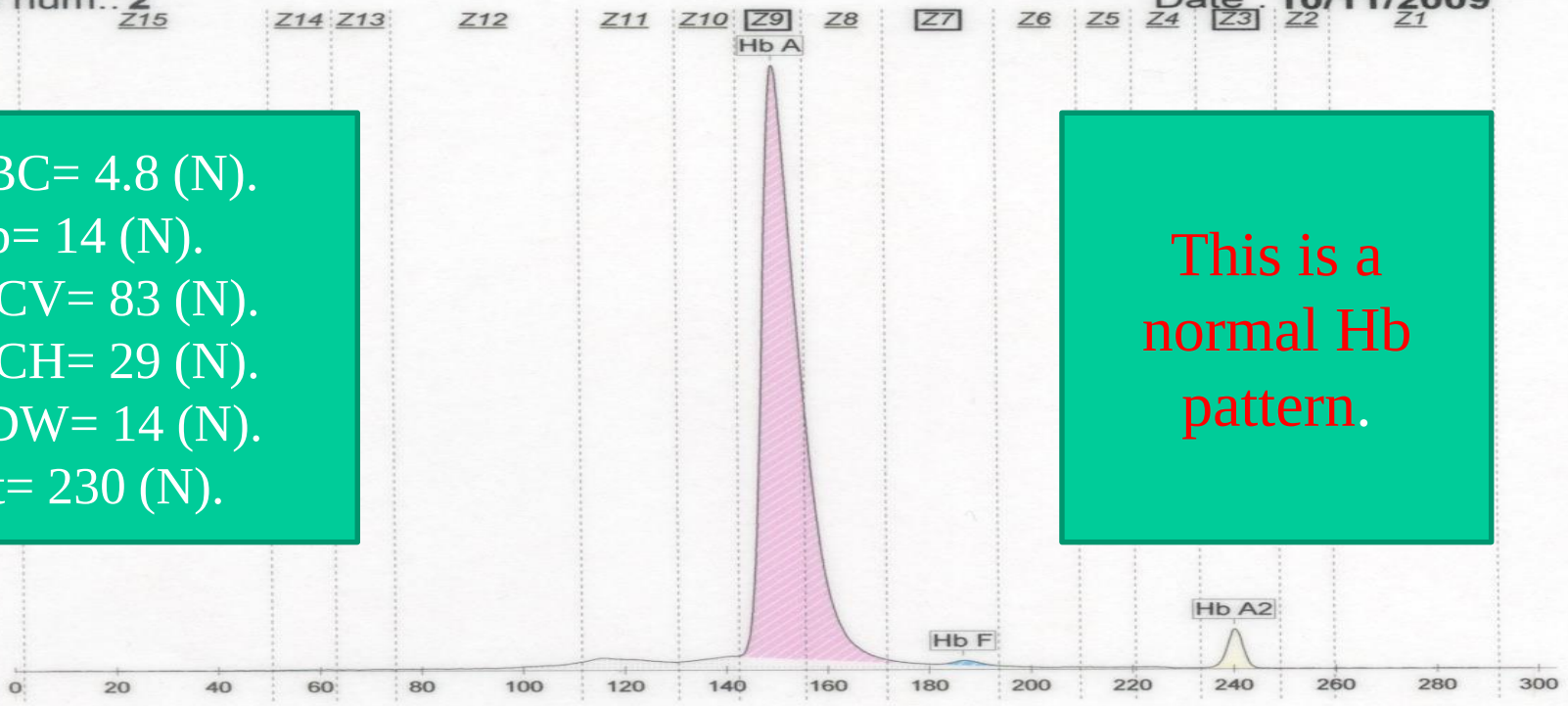
Hb Electrophoresis

Hospital No.: **933376**

ID : **061773**

Sample num.: **2**

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	

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Hb Electrophoresis

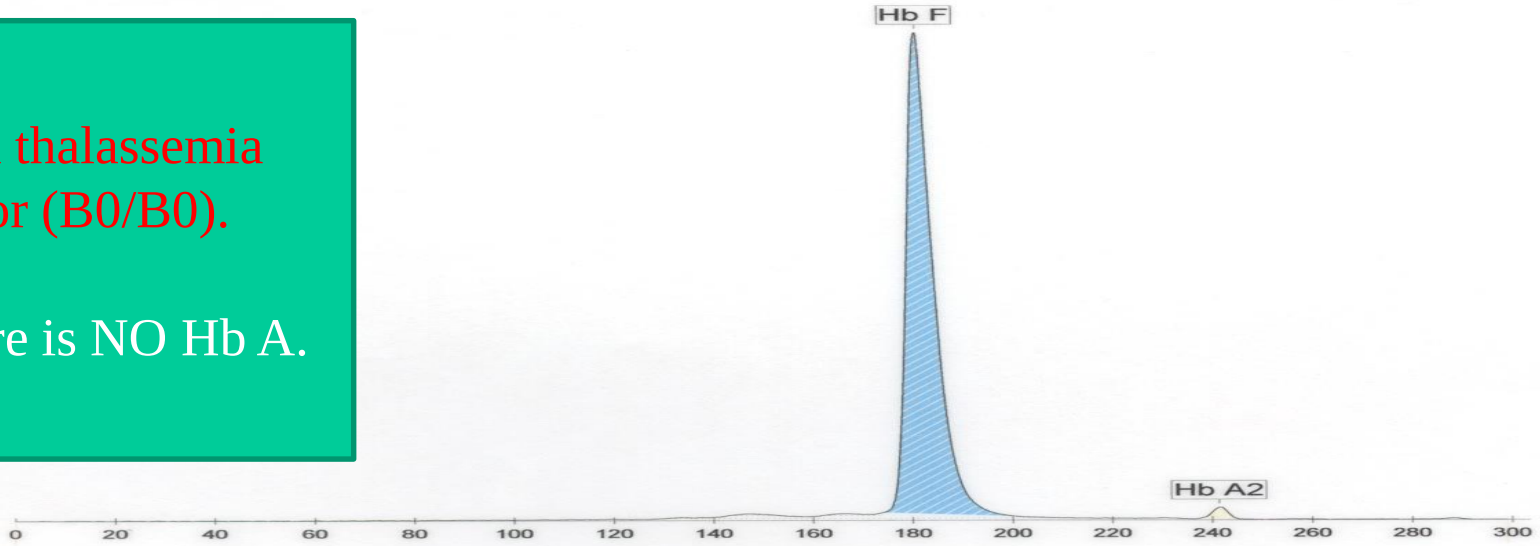
INSTRUMENT ID : KKUH : 24509

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

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Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

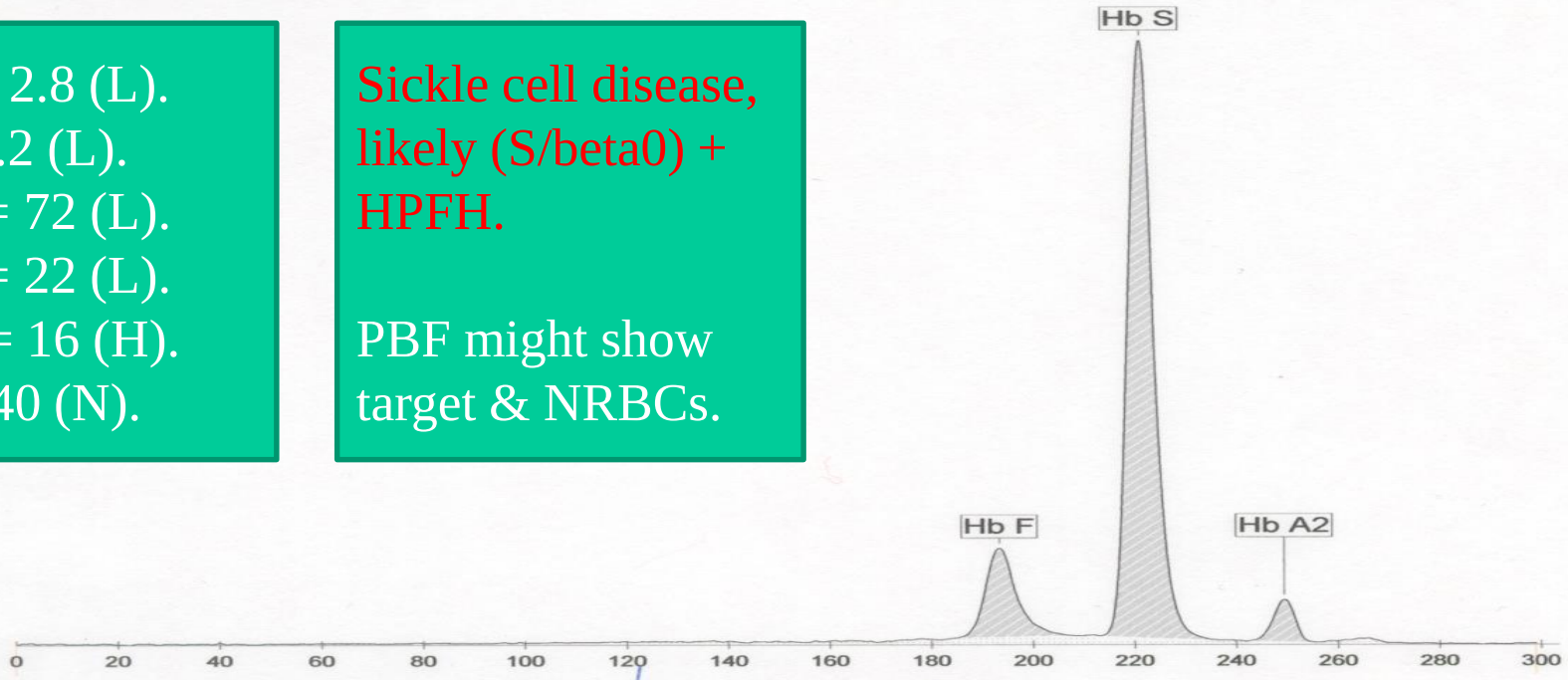
Hospital No.: 233095 ID : 063478

Sample No 20 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	

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Hb Electrophoresis

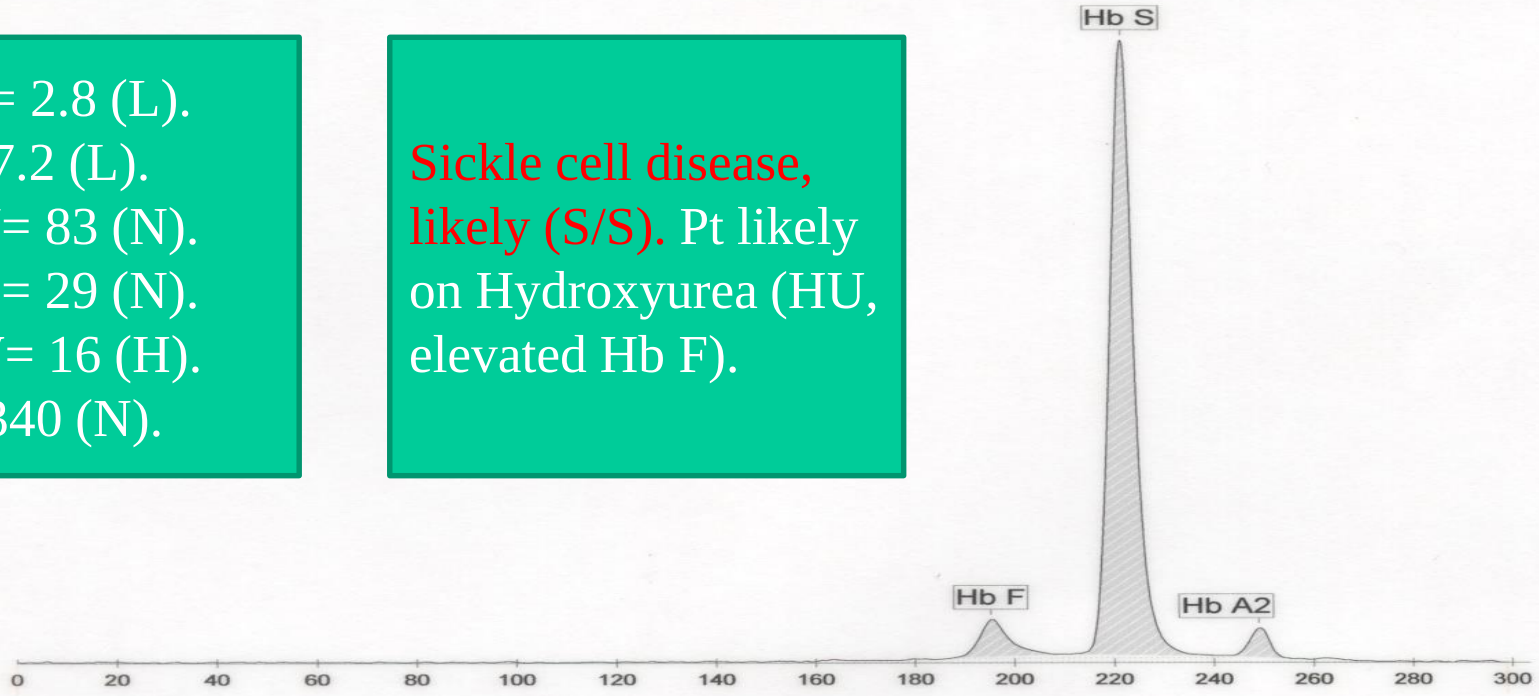
INSTRUMENT ID : KKUH : 24509

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	

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Hb Electrophoresis

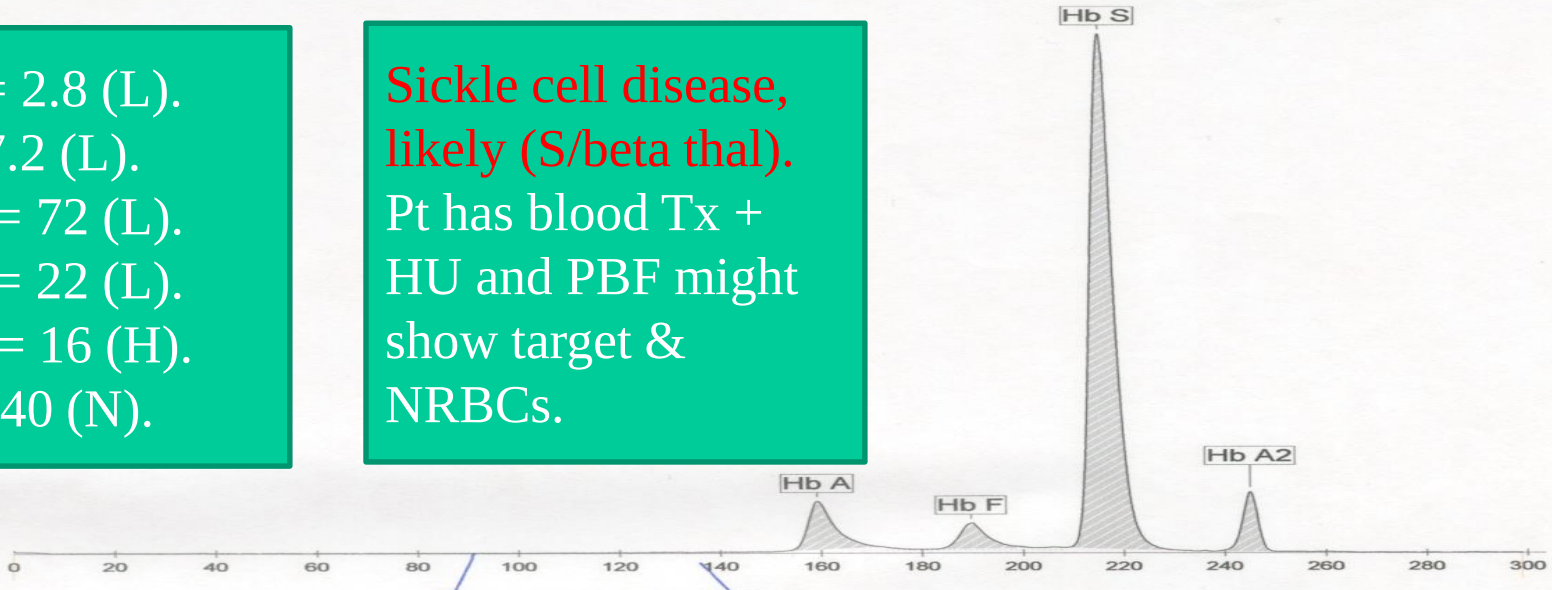
INSTRUMENT ID : KKUH : 24509

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

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Heamatology Unit

Hb Electrophoresis

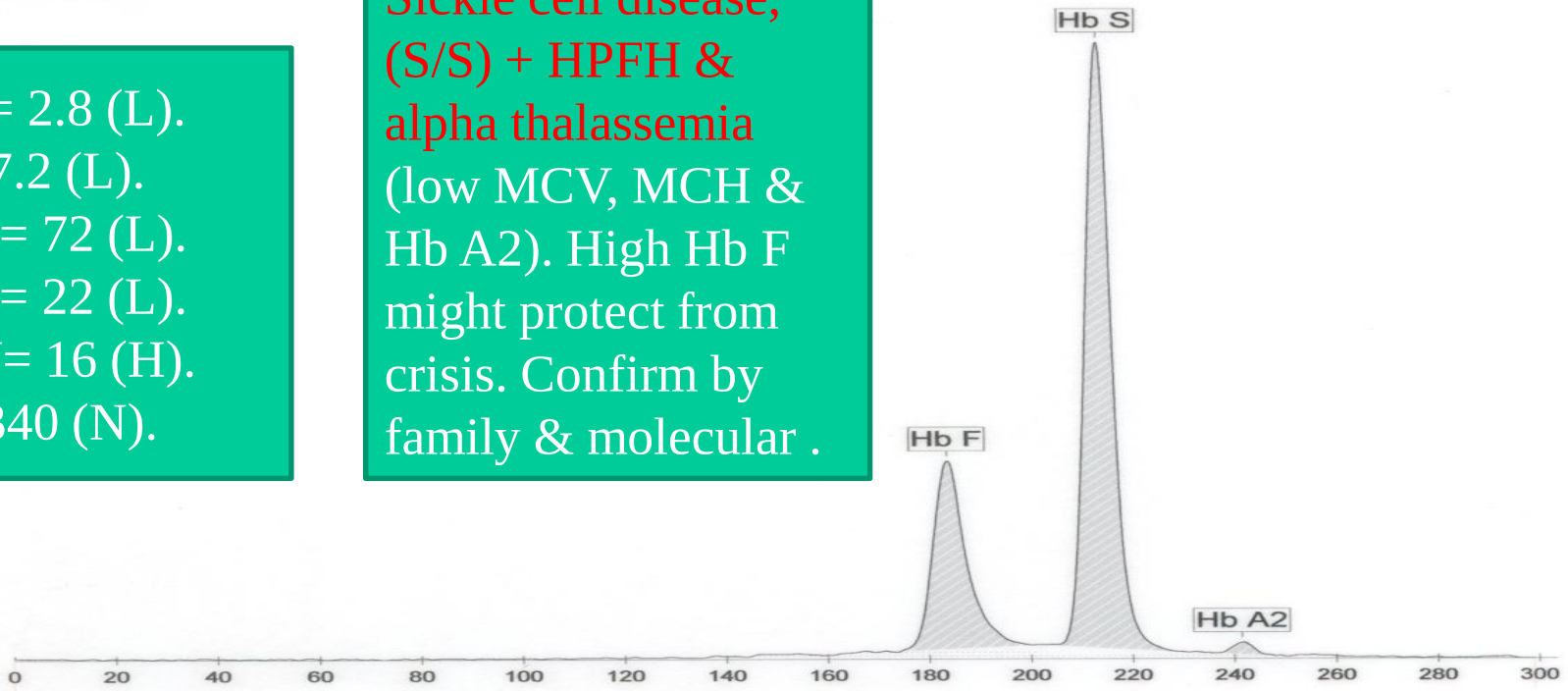
INSTRUMENT ID : KKUH : 24509

Hospital No.: 610043 ID : 064229

Sample No 52 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

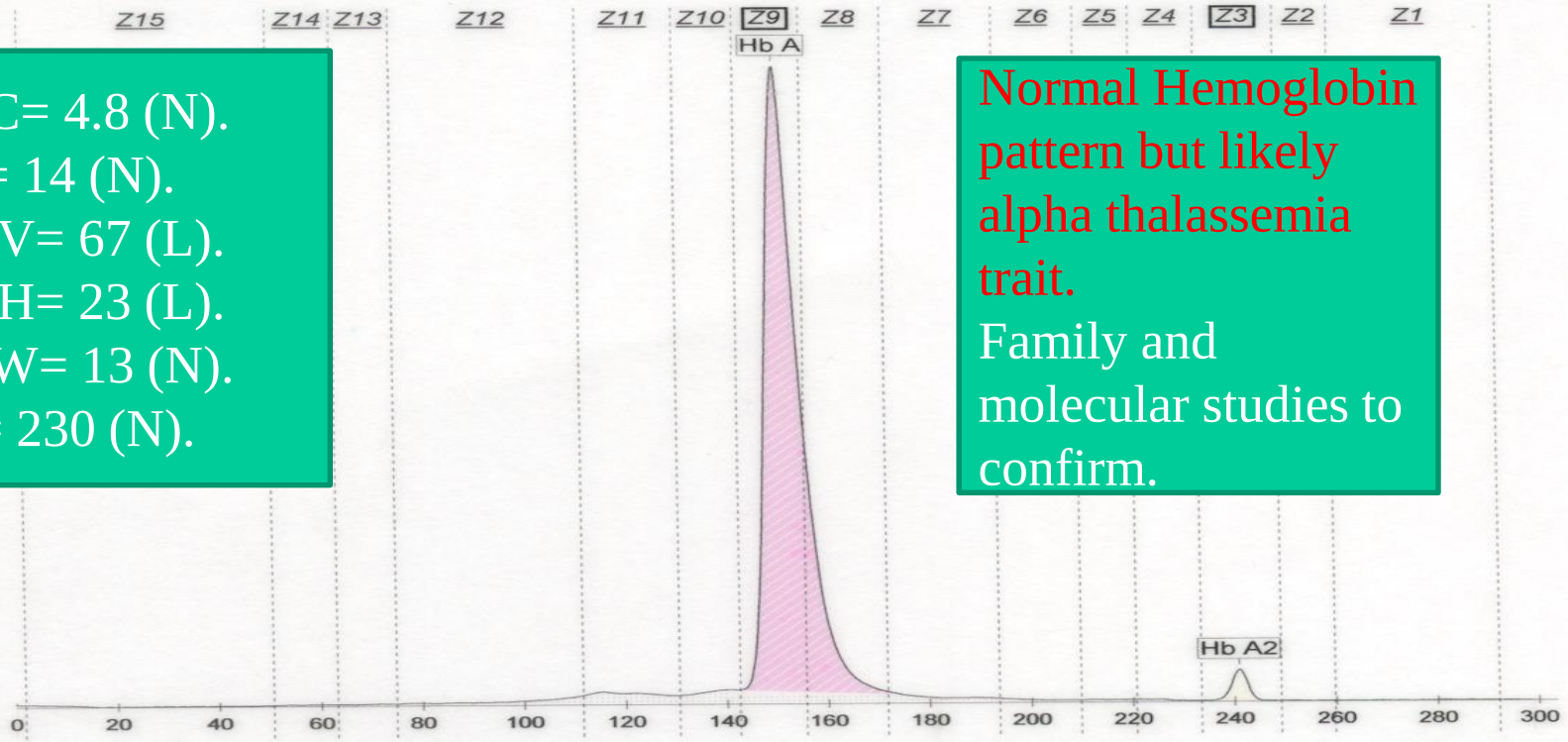
Hb Electrophoresis

INSTRUMENT ID : KKUH : 24509

Hospital No.: Rack: SEBIA Pos.: 2 ID : **ABDULLAH**

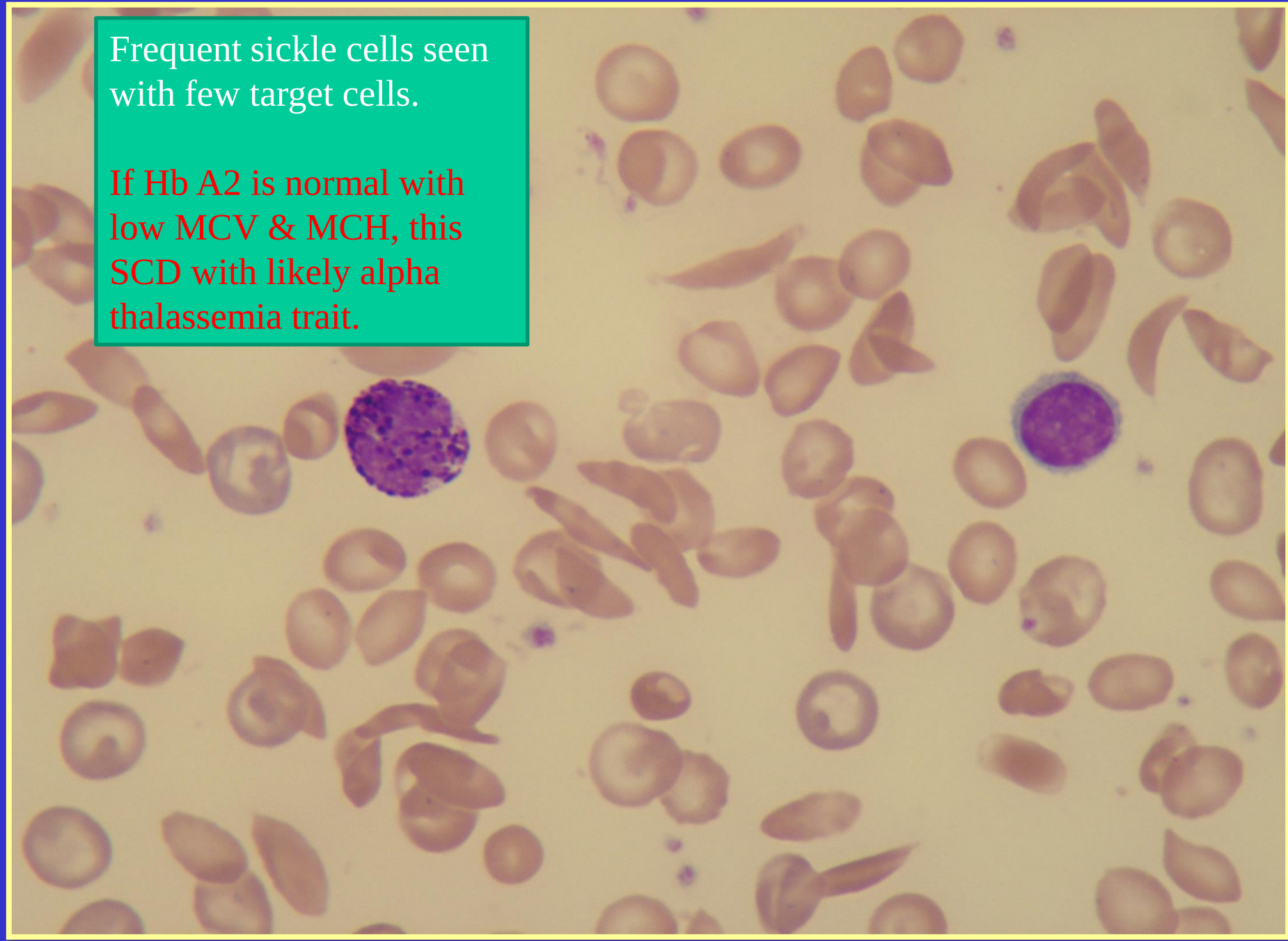
Sample No **20** Date : **19/05/2010**

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

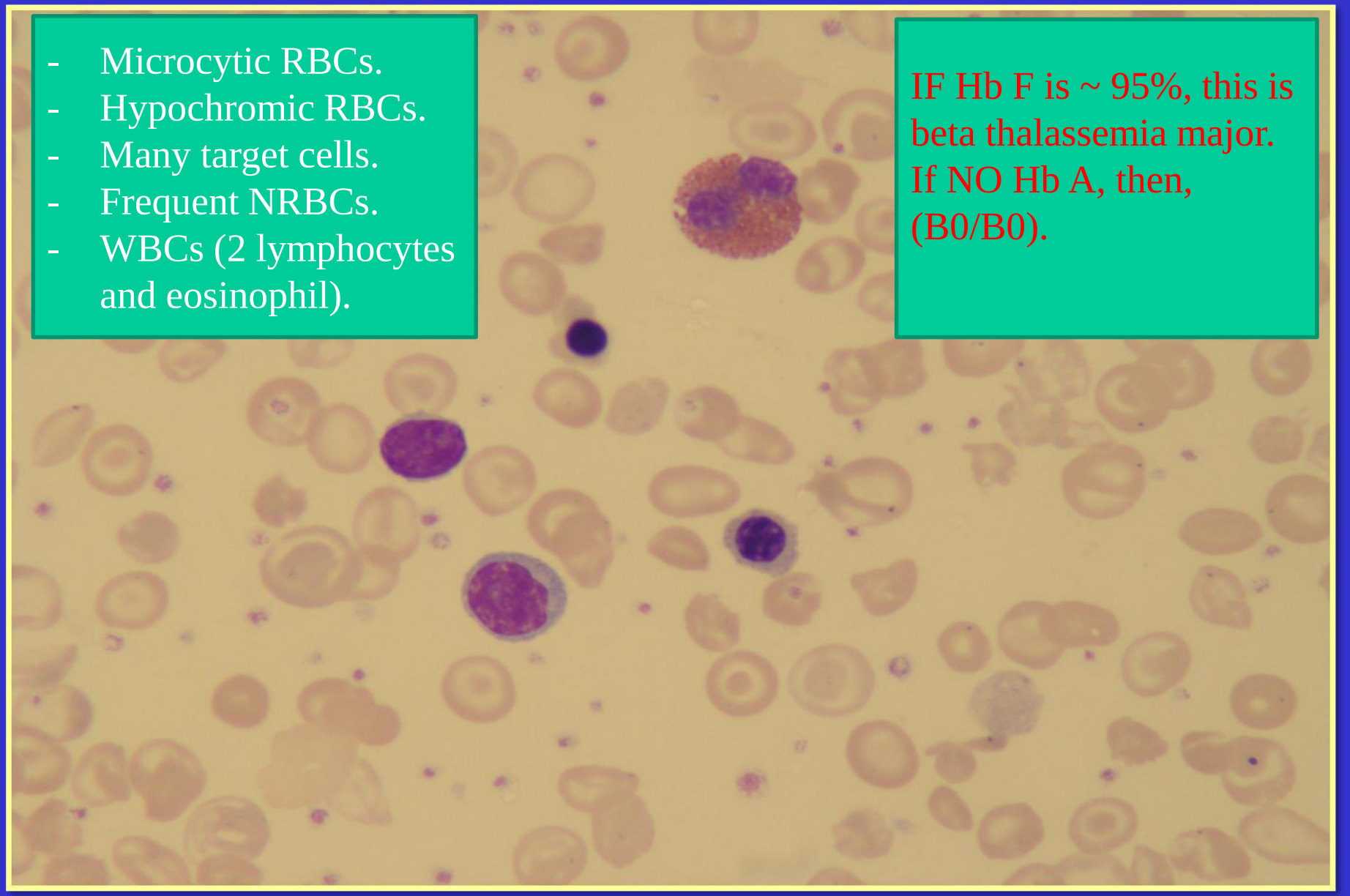


Normal Hemoglobin pattern but likely alpha thalassemia trait.
Family and molecular studies to confirm.

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 with likely alpha thalassemia trait.

Beta Thalassaemia Major



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

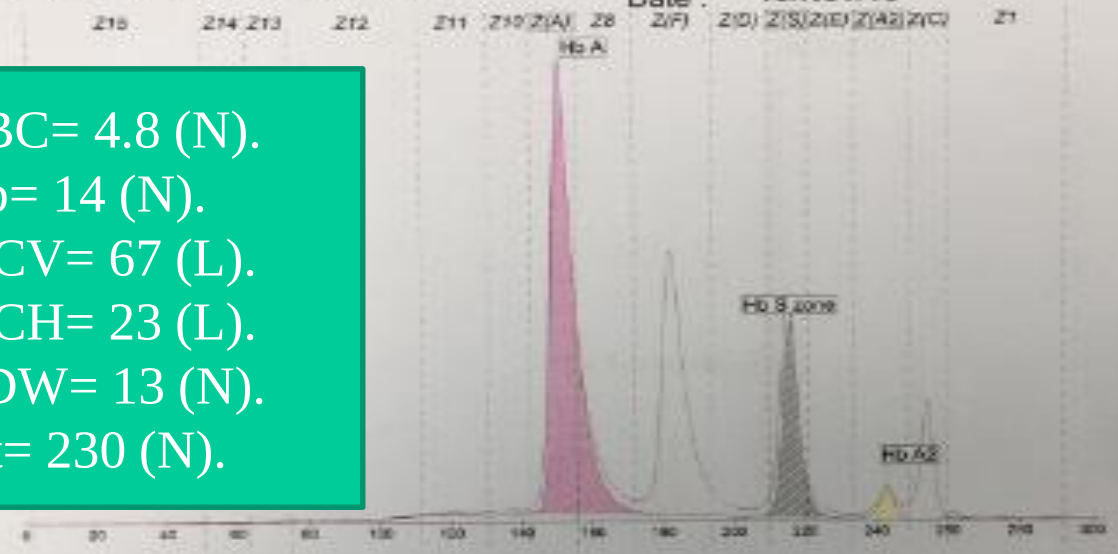
IF Hb F is ~ 95%, this is beta thalassemia major. If NO Hb A, then, (B0/B0).

MRN.: 10-09-53-11

Depart. K W-13 ANT

ACCESSION :011832300169A

Date : 19/Nov/18



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	

Comment : **Solubility Test: Positive (+ve)**

SOLUBILITY TEST

Signature

Sickle cell trait with likely alpha thalassemia.
Family and molecular studies to confirm.

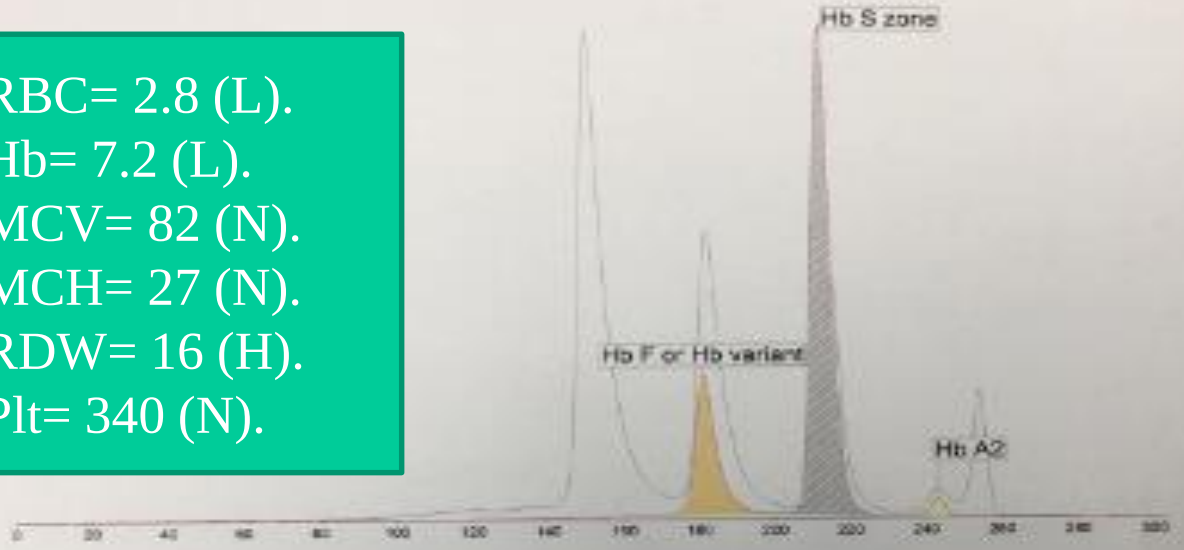
MRN.: 00-94-65-29

ACCESSION :011832204545A

Depart. K MED Clinic

Date : 19/Nov/18

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



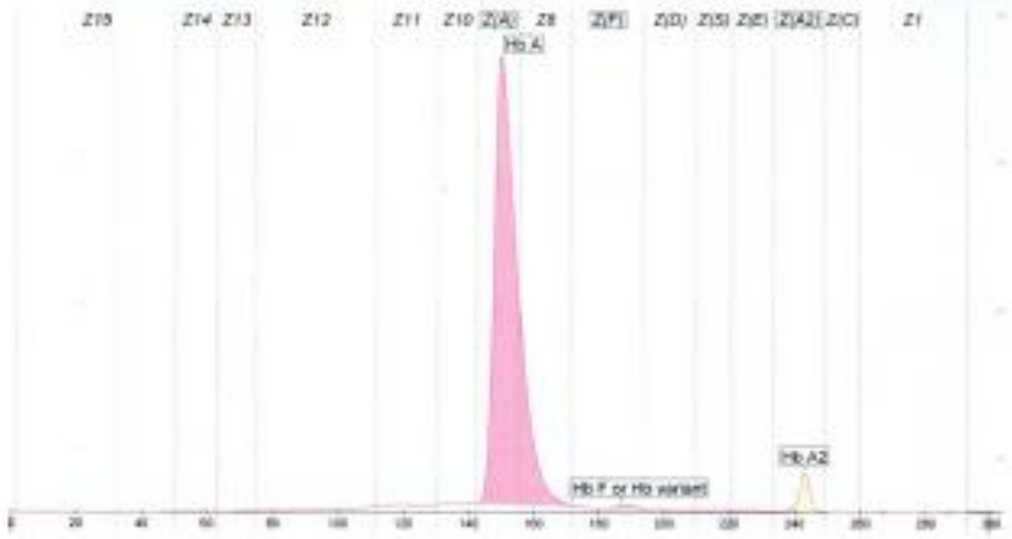
Fractions	%	Ref. %
Hb F or Hb variant	23.2	
Hb S zone	74.6	
Hb A2	2.2	

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 : 84.3
MCH : 28.4
RDW : 13.7
PH : 336

Comments

SOLUBILITY TEST

Signature


Thank you!
TESCHIO 99