MED439
KING SAUD UNIVERSITY

Hematology OSPE

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Dr's notes
Important
Extra notes
** Only in girls slide
** Only in boys slide

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Editing file

Golden Rules to Evaluate Hb 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. 3 different methods have their own issues (1-gel: alkaline or acid,2- HPLC (used a lot nowadays) & 3-capillary electrophoresis)
4. Family history and molecular tests are critical in difficult cases and to confirm the diagnosis.
5. As a physician, do not underestimate the medical history (blood transfusions) and clinical examination (looking for hepatosplenomegaly).
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 thalassaemia "combination").**
10. Alpha thalassemia reduced other abnormal Hb level , in trait state.

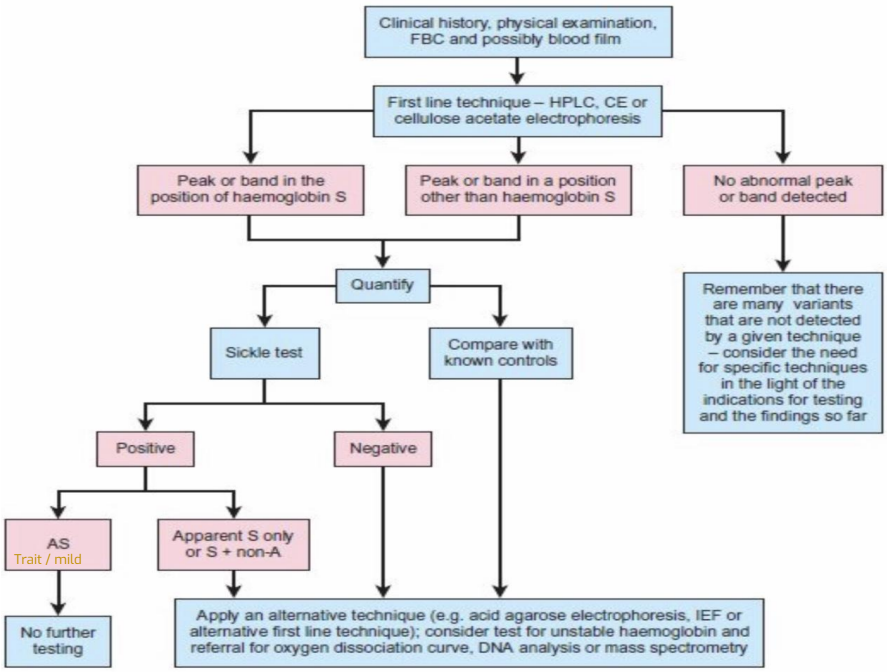
Doctor osama's note : usually alpha thalassemia is associated with minimal reduction in MCV and MCH. Unlike iron deficiency anemia which result in severe reduction of MCV , MCH .

★	Iron Deficiency Anemia	a-Thalassemia minor	B-Thalassemia Minor
MCV Mcv = mean corpuscular volume	↓	↓	↓
RDW RDW : red cell distribution width	↑	Normal	Normal
RBCs	↓	Normal	Normal
Peripheral smear	Microcytosis, hypochromia, **Pencil cell** (Elliptocytes)	**Target cells**	**Target cells**
Serum iron studies (also called iron profile)	↓Iron & ferritin [1] ↑ TIBC TIBC : total iron binding capacity	Normal/↑iron & ferritin (RBC tumover)	Normal/↑iron & ferritin (RBC tumover)
Response to iron supplementation	↑Hemoglobin	No improvement	No improvement
Hemoglobin electrophoresis (the golden test when suspecting thalasemia)	Normal	Normal	↑Hemoglobin A2

1) Ferritin is a blood protein that contains iron. A ferritin test helps your doctor understand how much iron your body stores. If a ferritin test reveals that your blood ferritin level is lower than normal, it indicates your body's iron stores are low and you have iron deficiency

★ Hb	Normal value
Hb A	96.8 - 97.8%
Hb F	=< 2%
Hb A2	1.5 - 3.5%

Not that imp



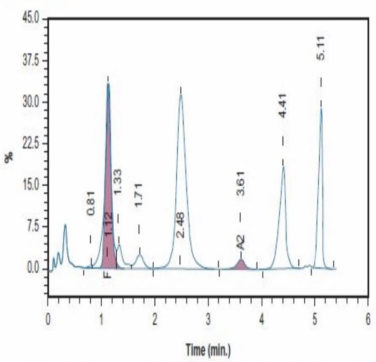


FIGURE 14-7 A mixture of haemoglobins separated by HPLC. From left to right the peaks are: injection artefact, post-translational modified haemoglobin F (double peak), haemoglobin F (pink), glycated haemoglobin A, other post-translationally modified haemoglobin A, haemoglobin A_2 (pink), haemoglobin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

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

We Compare results with the charter to diagnose the patient

TABLE 14-5

RESULTS OF LABORATORY INVESTIGATIONS IN INTERACTIONS OF HAEMOGLOBIN S AND α OR β THALASSAEMIA IN ADULTS

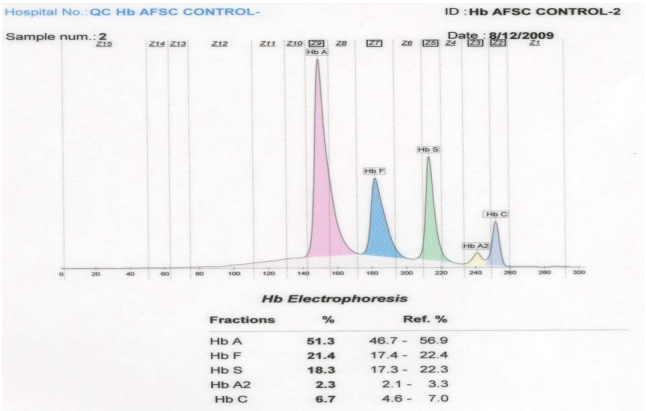
	MCV	% S	% A	% A_2	% F
AS	N	35–38	62–65	<3.5	<1
SS	N	88–93	0	<3.5	5–10
S/β^0 thalassaemia	L	88–93	0	>3.5	5–10
S/β^+ thalassaemia	L	50–93	3–30	>3.5	1–10
S/HPFH	N	65–80	0	<3.5	20–35
AS/α^+ thalassaemia	N/L	28–35	62–70	<3.5	<1
AS/α^0 thalassaemia	L	20–30	68–78	<3.5	<1
SS/α thalassaemia	N/L	88–93	0	<3.5	1–10

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

Dr osama's note : You can see from the table above that Beta thalassemia usually associated with high percentage of HbS. While alpha thalassemia associated with reduced percentage of HbS

This is a Quality Control sample (a synthetic sample) to ensure accurate results of the Hb electrophoresis

(normally there is a specific place for each peak, but no need to memorize the places + values)



Laboratory Investigations: Diagnostic Guide

To summarize :

	Sickle Cell Disease	Sickle Cell Trait	Alpha thalassemia	Beta Thalassemia Major	Beta Thalassemia Minor (Trait)
RBC	↓	↓	Normal or Slightly decreased	↓	Normal or Slightly decreased
MCV	Normal	Normal	↓	↓	↓
MCH	Normal	Normal	↓	↓	↓
HB A	Absent	Slightly decreased	Normal	Absent	↓
Hb A2	Normal	Normal	Normal or slightly decreased	Normal	↑4-8%
Hb F	↑	Normal	Normal	↑>80%	↑
Hb S	↑>45% Normally = zero	↑35-45% lower than 45% is sickle trait not disease	Absent	Absent	Absent

Laboratory Investigations (1): Hemoglobin Electrophoresis

Diagram	CBC	Hb electrophoresis	Findings	Most likely diagnosis	Further investigations For confirming the diagnosis
A	RBC= 4.8 (N) Hb= 14 (N) MCV= 83 (N) MCH= 29 (N) RDW= 14 (N) Plt= 230 (N).	**Hb A:** 96.7 **Hb F:** 0.5 **Hb A2:** 2.8	**Hb A:** normal **Hb F:** normal **Hb A2:** normal	**Normal Hb pattern**	NONE
B	Hb= 9.8 MCV= 73 NRBC=34	**Hb F:** 98.5 **Hb A2:** 1.5	**Hb A:** absent **Hb F:**very high **Hb A2:** normal	**Beta thalassemia major (BO/BO).**	Family and molecular studies
C	RBC= 2.8 (L). Hb= 7.2 (L). **MCV= 72 (L).** **MCH= 22 (L).** RDW= 16 (H). Plt= 340 (N).	**Hb F:** 14.7 **Hb S:** 80.5 **Hb A2:** 4.8	**Hb A:** absent **Hb F:** high **Hb A2:** high **Abnormal Hb:** Hb S very high	**Sickle cell disease, likely (S/beta0) + HPFH.** PBF might show target & NRBCs.	
D	RBC= 2.8 (L). Hb= 7.2 (L). **MCV= 83 (N).** **MCH= 29 (N).** RDW= 16 (H). Plt= 340 (N).	**Hb F:** 6.5 **Hb S:** 89.9 **Hb A2:** 3.6	**Hb A:** absent **Hb F:** high **Hb A2:** normal **Abnormal Hb:** Hb S very high	Pure Sickle cell disease, likely (S/S). Pt likely on Hydroxyurea (HU, elevated Hb F).	
E	RBC= 2.8 (L). Hb= 7.2 (L). **MCV= 72 (L).** **MCH= 22 (L).** RDW= 16 (H). Plt= 340 (N).	**Hb A:** 8.7 **Hb F:** 4.9 **Hb S:** 80.1 **Hb A2:** 6.3	**Hb A:** low - n **Hb F:** high **Hb A2:** high **Abnormal Hb:** Hb S very high	Sickle cell disease, likely (S/beta thal). Pt has blood Tx + HU and PBF might show target & NRBCs.	

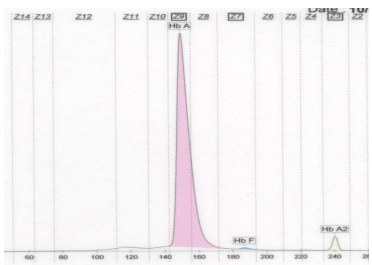


Diagram A

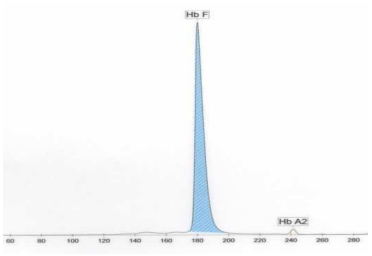


Diagram B

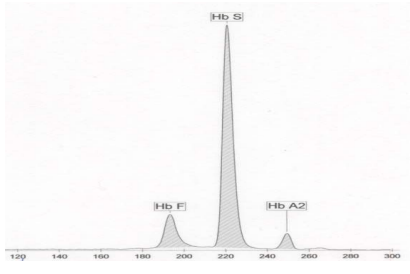


Diagram C

What is the name of the Test ?
HB electrophoresis

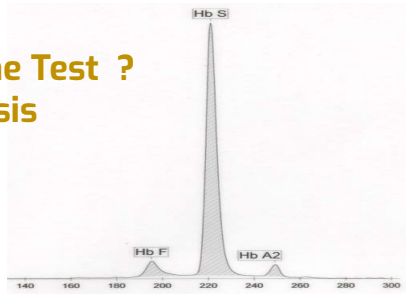


Diagram D

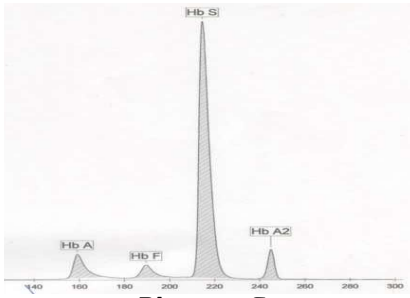


Diagram E

Laboratory Investigations (1): Hemoglobin Electrophoresis

Diagram	CBC	Hb electrophoresis	Findings	Most likely diagnosis	Further investigations For confirming the diagnosis
★ F	RBC= 2.8 (L) Hb= 7.2 (L) **MCV= 72 (L)** **MCH= 22 (L)** RDW= 16 (H) Plt= 340 (N).	**Hb F:** 28.1 **Hb S:** 70.8 **Hb A2:** 1.1	**Hb A:** absent **Hb F:**very high **Hb A2:** low **Abnormal Hb:** Hb S very high	**Sickle cell disease, (S/S) + HPFH & alpha thalassemia** (low MCV, MCH & Hb A2).High Hb F might protect from crisis.	Family and molecular studies
★ G	RBC= 4.8 (N) Hb= 14 (N) **MCV= 67 (L)** **MCH= 23 (L)** RDW= 13 (N) Plt= 230 (N).	**Hb A:** 97.7 **Hb A2:** 2.3	**Hb A:** normal **Hb F:** absent **Hb A2:** normal	Normal Hemoglobin pattern but likely alpha thalassemia trait.	
H	RBC= 4.8 (N) Hb= 14 (N). **MCV= 67 (L)** **MCH= 23 (L)** RDW= 13 (N) Plt= 230 (N).	**Hb A:** 73.9 **Hb S:** 22.6 **Hb A2:** 3.5 Solubility test +	**Hb A:** low **Hb F:** absent **Hb A2:** normal **Abnormal Hb:** Hb S high (trait)	**Sickle cell trait with likely alpha thalassemia.** **Solubility test is +ve, which is a strong indication of alpha thalassemia**	
I	RBC= 2.8 (L) Hb= 7.2 (L) **MCV= 82 (N)** **MCH= 27 (N)** RDW= 16 (H) Plt= 340 (N).	**Hb F:** 23.2 **Hb S:** 74.6 **Hb A2:** 2.2 Solubility test +	**Hb A:** absent. **Hb F:**very high **Hb A2:** normal **Abnormal Hb:** Hb S high	**Sickle cell disease, (S/S) + HPFH.** - (normal MCV, MCH & Hb A2). High Hb F might protect from crisis.	
J	RBC= 4.4 Hb= 12.5 **MCV=84.9** **MCH= 28.4** RDW= 13.7 Plt= 336 WBC = 3.1(low)	**HbA:**95.5< **Hb F:** 0.8> **HbA2:**3.7>	**Hb A:** normal **Hb F:** normal **Hb A2:** normal	**Normal results even with slightly elevated Hb A2** (NOT beta thalassemia trait).	

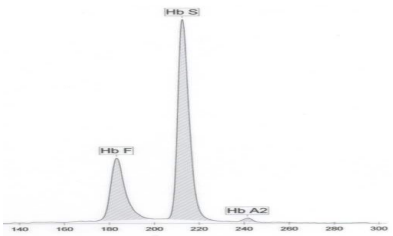


Diagram F

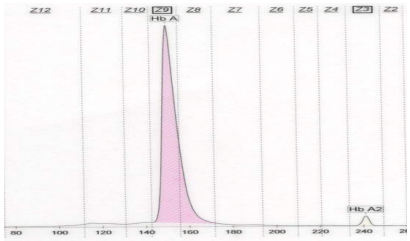


Diagram G

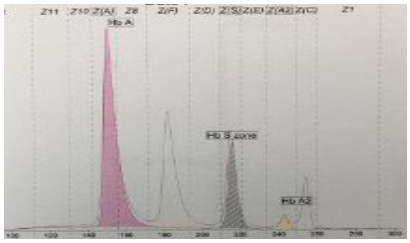


Diagram H

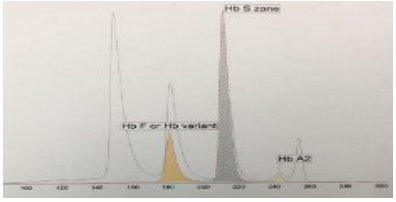


Diagram I

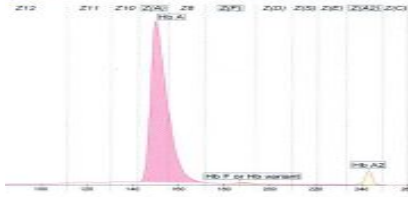


Diagram J

Laboratory Investigations (1): Hemoglobin Electrophoresis

Same pictures, just bigger size

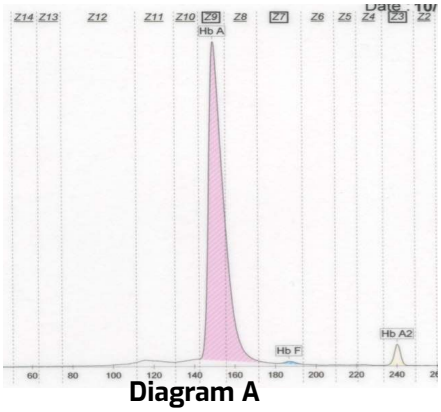


Diagram A

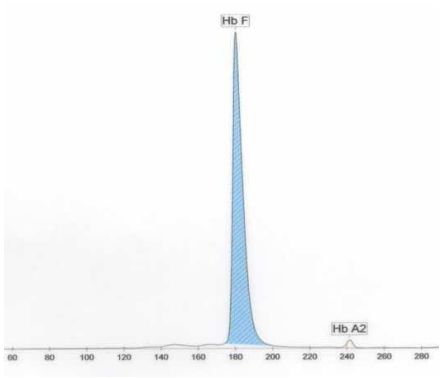


Diagram B

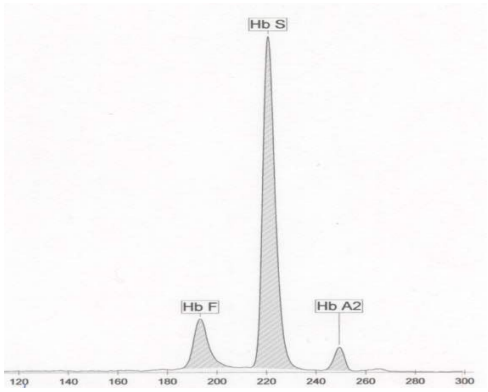


Diagram C

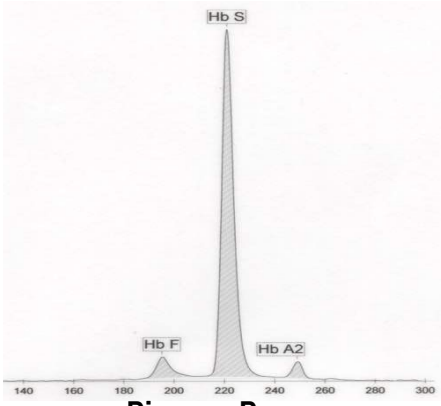


Diagram D

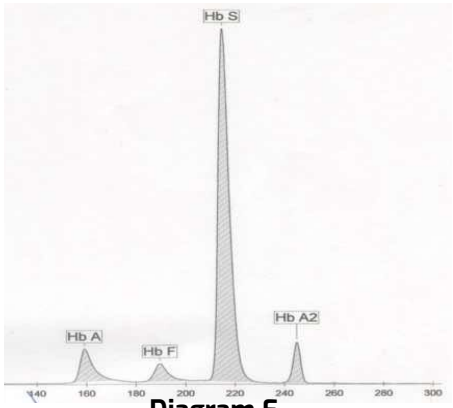


Diagram E

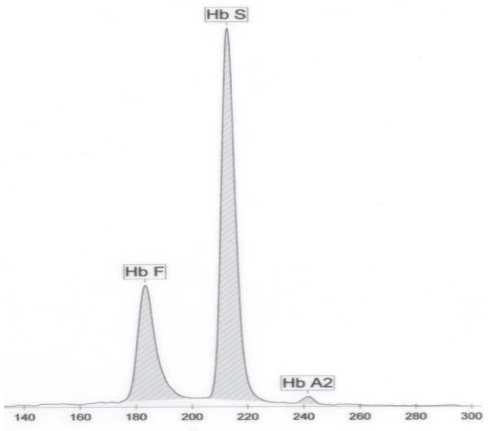


Diagram F

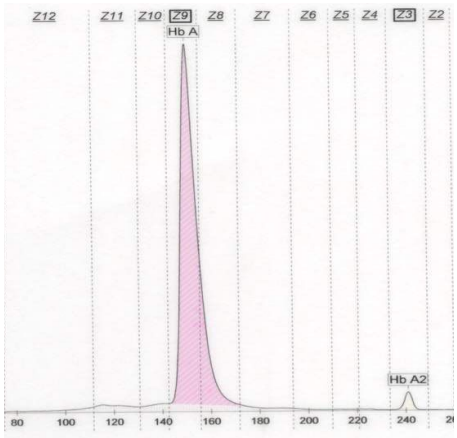


Diagram G

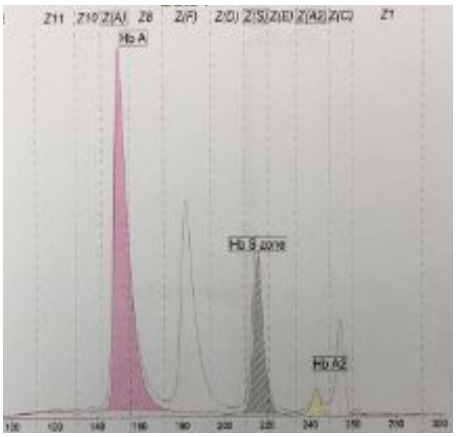


Diagram H

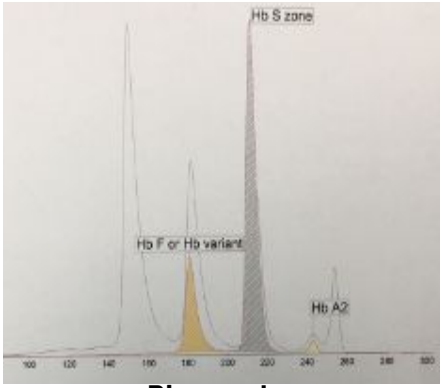


Diagram I

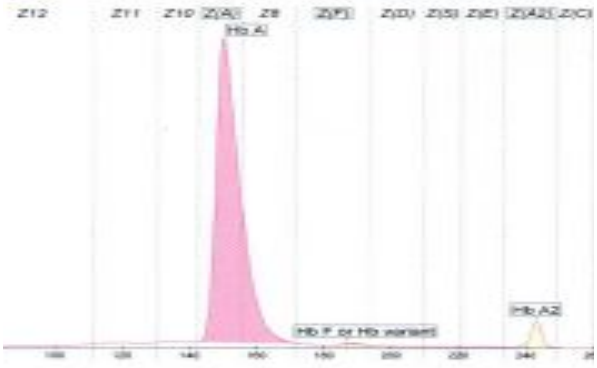
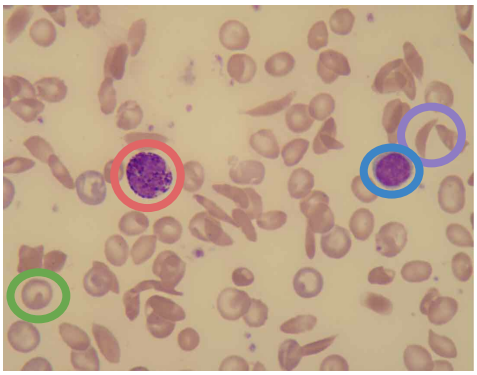
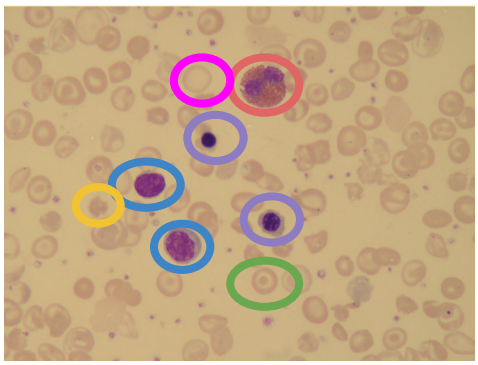
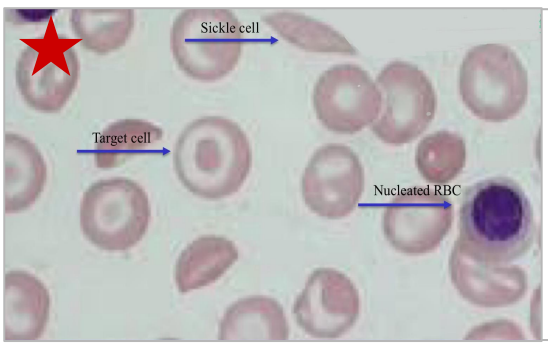


Diagram J

Laboratory Investigations (2): Blood Smear/Film

	A	B
Microscopy	★ **What is the name of the Test ? Blood film** 	
Findings	-Sickled cells -Target cells -Basophil -Lymphocyte	-Microcytic RBCs. -Hypochromic RBCs. -Many target cells. -Frequent NRBCs (nucleated red blood cells). -WBCs (2 lymphocytes and eosinophil).
Most likely diagnosis	If Hb A2 is normal with low MCV & MCH: **Sickle cell disease maybe (S/S) with likely an alpha thalassemia trait** Whenever you see sickled cells it means sickle cell disease	If Hb F is ~ 95%: **beta thalassemia major**. If no Hb A : **beta thalassemia** (B0/B0)
Further investigations	- molecular studies - Family studies - Hb electrophoresis	★ To diagnose blood diseases (in order) 1- CBC 2- Blood film / smear 3- HB electrophoresis
		★ To confirm your diagnosis: Family and molecular studies



What can you see ?

1- Many target cells
2- nucleated RBCs
3- sickle cell seen.

If Hb A2 is elevated with low MCV & MCH
(this SCD likely (S/Beta thal) genotype.)
even if we don't say that u can know the diagnosis from morphology

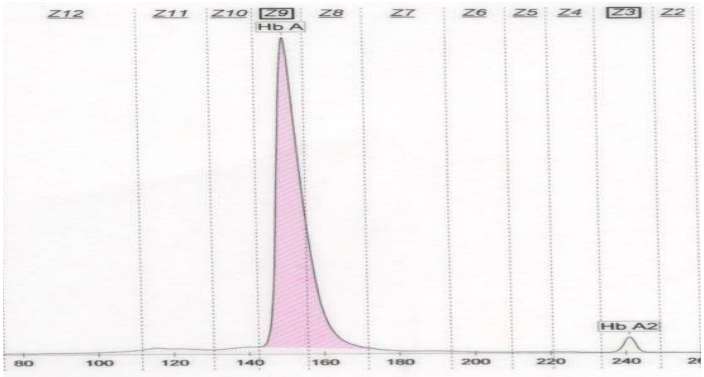
Helpful Questions For practicing

Extra

#438

Practice (1)

A 16-year old Indonesian male with no known prior medical history came to the hospital complaining of mild fatigue accompanied with shortness of breath during rest,Initial clinical examination shows a slightly pale appearance.A precautionary CBC test was conducted and the results were as following : RBC= 4.8,Hb= 14,MCV= 67,MCH= 23,RDW=13 ,Platelet count= 230.A Figure was attached below with the following results:Hb A: 97.7% , Hb A2: 2.3% .Based on these results and the figure below answer the following questions:



Q1:What is the name of test shown above:

Hemoglobin Electrophoresis

Q2:Comment on the findings based on the test results shown on the figure:

1-Normal Hb A , Normal value (96.8-97.8)

2-Normal Hb A2 , Normal value (1.5-3.5)

3-Absent Hb F , Normal Value (<2)

Q3:Comment on the CBC results shown:

Hypochromic Microcytic Anemia with Normal Hb and RBC levels

Q4:What is the most likely diagnosis based on the findings:

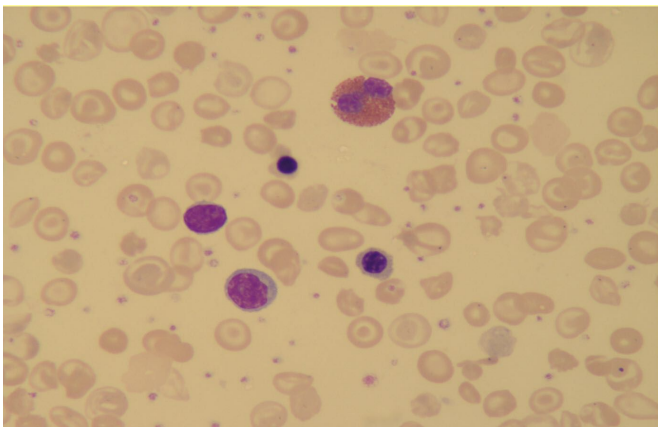
Normal hemoglobin pattern but likely an Alpha thalassemia trait

Q5:How to confirm the diagnosis:

1-Molecular studies 2-Family studies

Practice (2)

A 22-year old female presents to the ER with extreme fatigue accompanied with proximal weakness ,Initial clinical examination shows pale appearance.A CBC test was done and the results show the following : Hb 9.8, MCV 73, NRBC 34 ,The physician acknowledged the Anemic clinical pattern and ordered A Hb Electrophoresis test to be done and the results were as following: Hb A2: 1.5, Hb F 98.5.A **Blood smear** was done and the results were shown on the figure below.Based on these results and the figure below answer the following questions:



Q1:Comment on the results of Hb Electrophoresis:

1-High Hb F level

2-Normal Hb A2 level

3- Absence of Hb A

Q2:Comment on the findings of the Blood smear test shown:

1-Microcytic RBCs

2-Hypochromic RBCs

3-Many NRBCs

4-Many Target Cells

5-Lymphocytes

6-Eosinophils

Q3:What is the most likely diagnosis based on the findings:

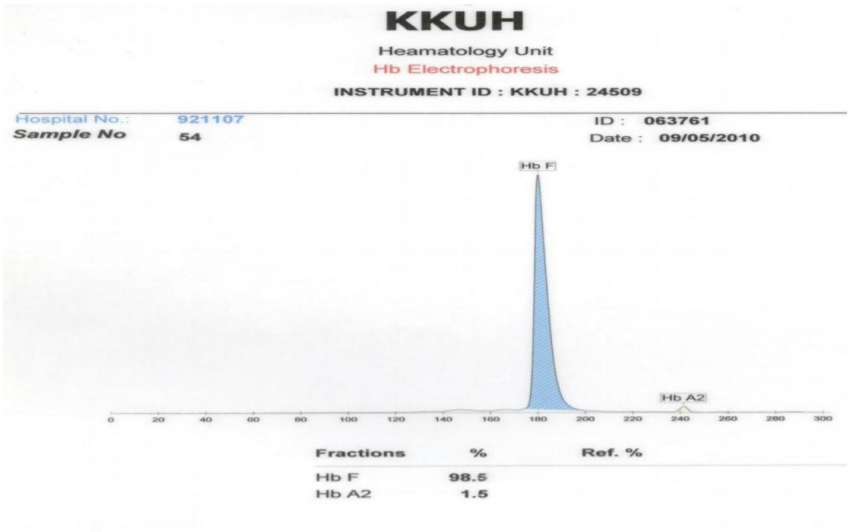
Beta thalassemia Major (B0/B0)

Q4:How to confirm the diagnosis:

1-Molecular studies

2-Family studies

#436



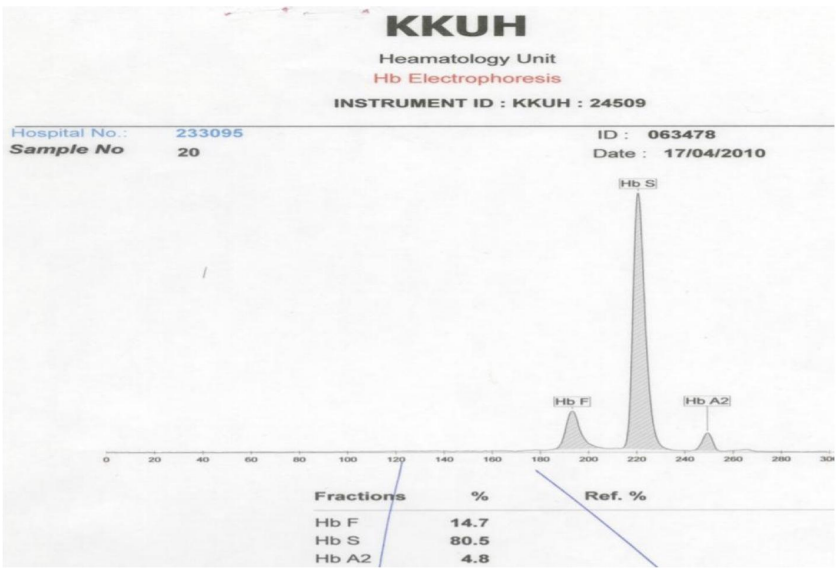
What is the name of test performed?
Hemoglobin electrophoresis

What are the findings?
Hb A: absent
Hb F: very high
Hb A2: normal

What is the most likely diagnosis?
High persistent HB F disease **OR** Normal fetus

What further investigations will you order?

Genetic study
Family study



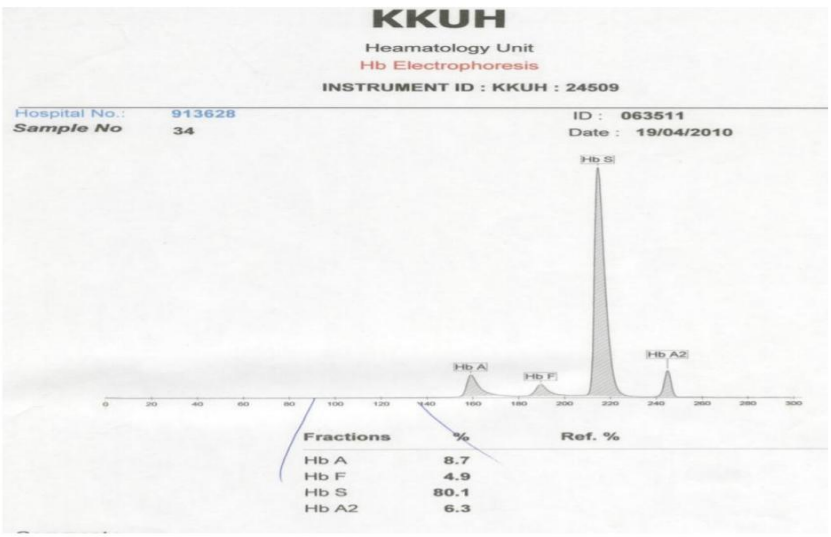
What is the name of test performed?
Hemoglobin electrophoresis

What are the findings?
Hb A: absent
Hb F: high
Hb A2: high
Abnormal Hb: Hb S high → sickle cell disease

What is the most likely diagnosis?
Sickle cell anemia disease with beta thalassemia

What further investigations will you order?

Genetic study
Family study



What is the name of test performed?
Hemoglobin electrophoresis

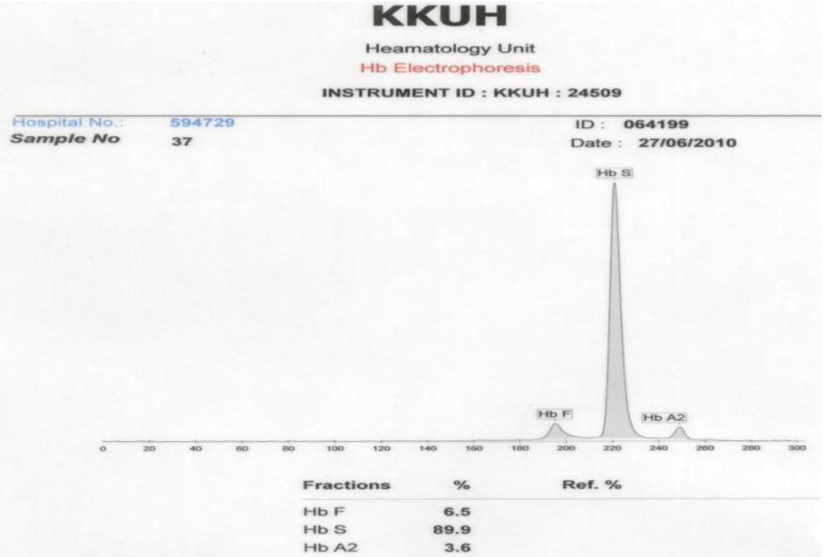
What are the findings?
Hb A: very low
Hb F: high
Hb A2: high
Abnormal Hb: Hb S high → sickle cell disease

What is the most likely diagnosis?
Sickle cell anemia disease with beta thalassemia
(with Hb A from blood transfusions)

What further investigations will you order?

Genetic study
Family study

#436

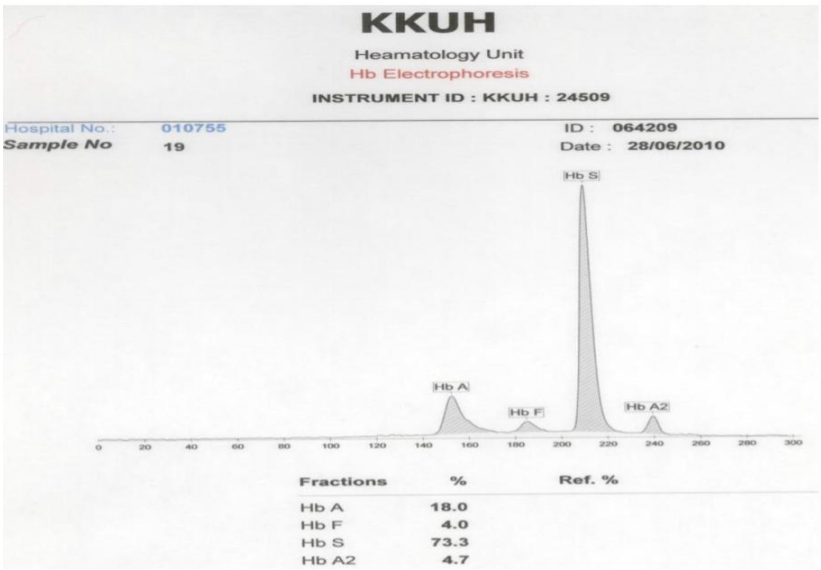


What is the name of test performed?
Hemoglobin electrophoresis

What are the findings?
Hb A: absent
Hb F: high
Hb A2: normal
Abnormal Hb: Hb S high → sickle cell disease

What is the most likely diagnosis?
Sickle cell anemia disease

What further investigations will you order?
Genetic study
Family study

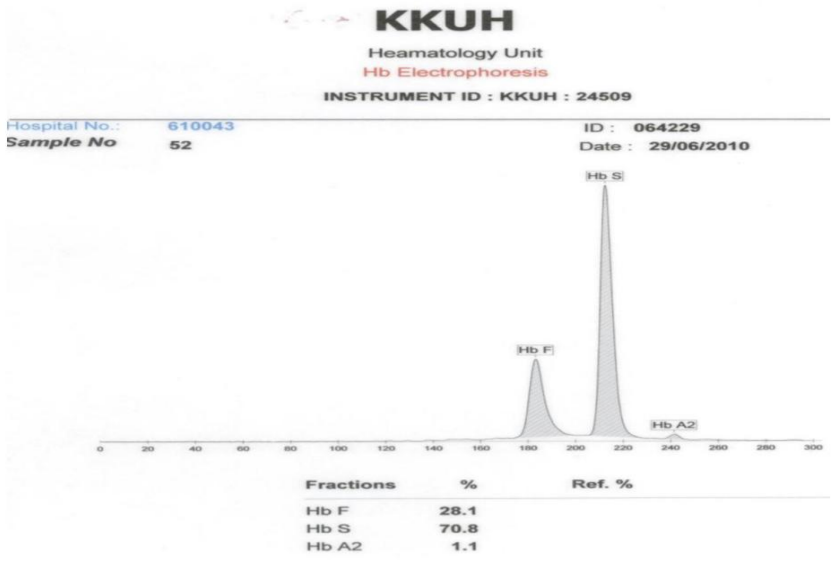


What is the name of test performed?
Hemoglobin electrophoresis

What are the findings?
Hb A: very low
Hb F: high
Hb A2: high
Abnormal Hb: Hb S high → sickle cell disease

What is the most likely diagnosis?
Sickle cell anemia disease with beta thalassemia
(with Hb A from blood transfusions)

What further investigations will you order?
Genetic study
Family study



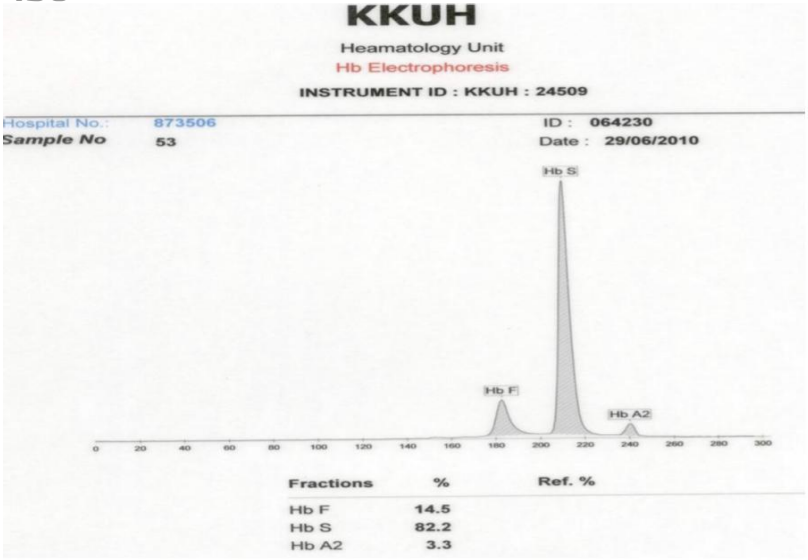
What is the name of test performed?
Hemoglobin electrophoresis

What are the findings?
Hb A: absent
Hb F: high
Hb A2: low
Abnormal Hb: Hb S high → sickle cell disease

What is the most likely diagnosis?
Sickle cell anemia disease with alpha thalassemia
with high Hb F

What further investigations will you order?
Genetic study
Family study

#436

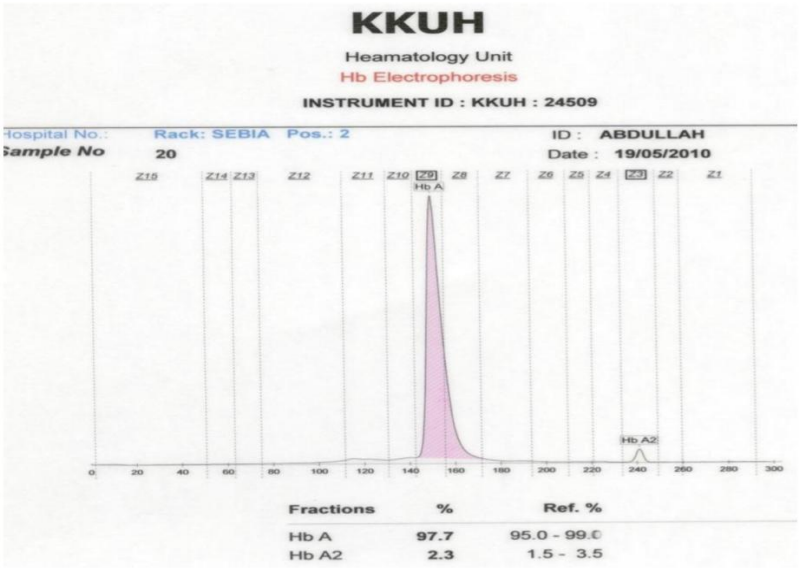


What is the name of test performed?
Hemoglobin electrophoresis

What are the findings?
Hb A: absent
Hb F: high
Hb A2: normal
Abnormal Hb: Hb S high → sickle cell disease

What is the most likely diagnosis?
Sickle cell anemia disease, with high Hb F

What further investigations will you order?
Genetic study
Family study

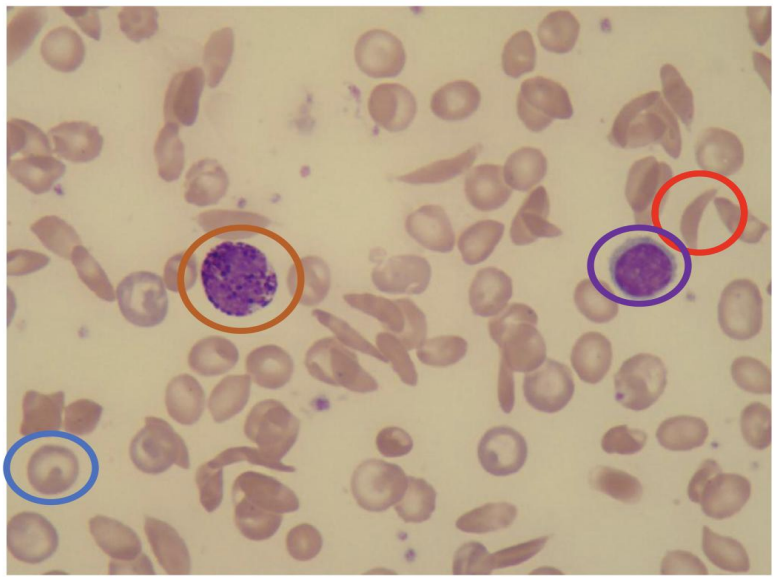


What is the name of test performed?
Hemoglobin electrophoresis

What are the findings?
Hb A: normal
Hb F: absent
Hb A2: normal

What is the most likely diagnosis?
Normal electrophoresis (even with HbF absence)

What further investigations will you order?
NONE



What is the name of test performed?
Blood smear/film

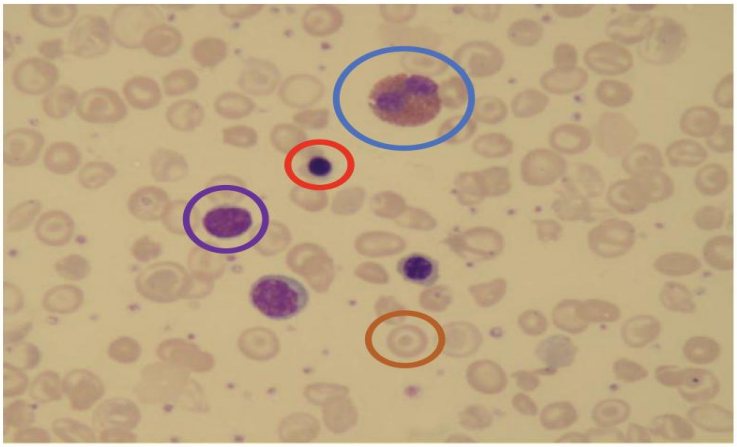
What are the findings?
Sickled cells
Target cells
Basophil
Lymphocyte

What is the most likely diagnosis?
Sickle cell anemia disease

What further investigations will you order?

Hemoglobin electrophoresis
Genetic study
Family study

#436



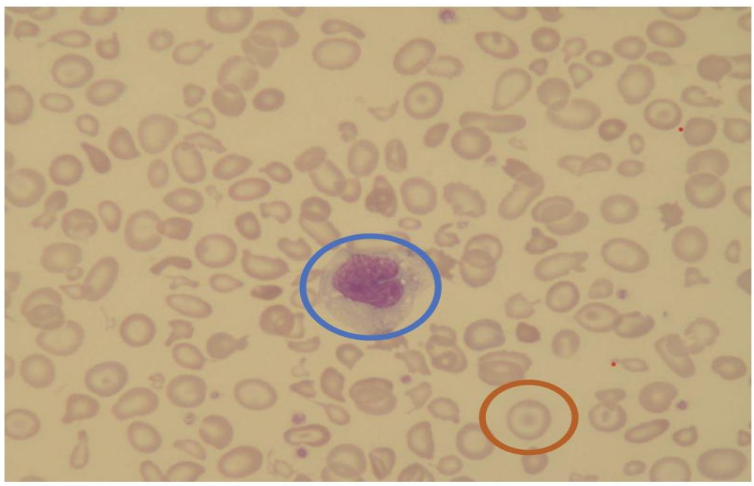
What is the name of test performed?
Blood smear/film

What are the findings?
Nucleated RBCs
Hypochromsia
Microcytosis
Esinophils - lymphocytes
Anisocytosis (variation in size)
Poikilocytosis (variation in shape).
Target cells

What is the most likely diagnosis?
Beta thalassemia major

What further investigations will you order?

Hemoglobin electrophoresis
Genetic study
Family study



What is the name of test performed?
Blood smear/film

What are the findings?
Hypochromsia
Microcytosis
Monocytes
Anisocytosis (variation in size)
Poikilocytosis (variation in shape).
Target cells

What is the most likely diagnosis?
Alpha thalassemia

What further investigations will you order?

Hemoglobin electrophoresis
Genetic study
Family study