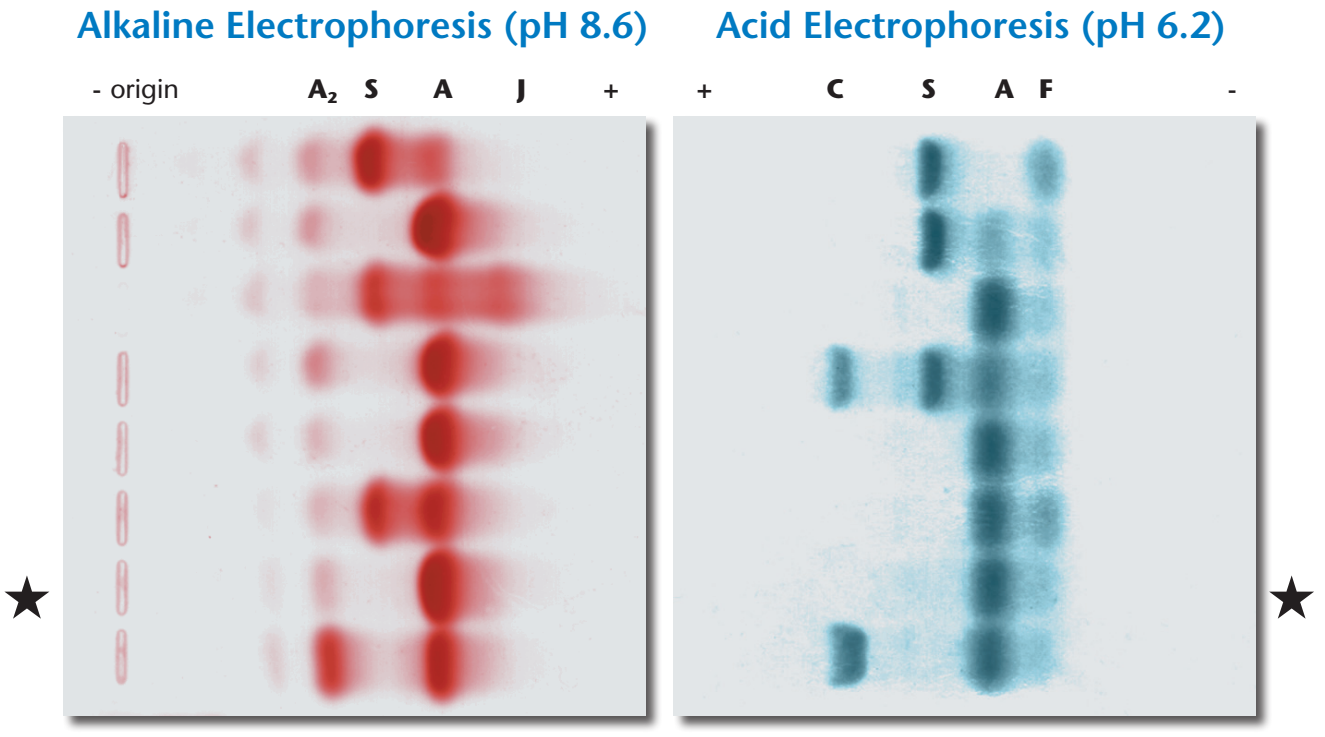
Case 1 Discussion



Interpretation

On alkaline electrophoresis, there is a band in the A position and a minor band in the A_2 position. No other abnormal bands are present. On acid electrophoresis, there is a band in the A position.

Diagnosis

Normal adult.

Performance

Normal specimens have been used numerous times in the survey. Typically, >95% of laboratories correctly identify a normal specimen.

Discussion

This sample is included to illustrate the pattern seen on a normal specimen. On alkaline electrophoresis, there is a major band in the A position and a minor band in the A_2 position. There is also a minor band seen cathodal to the A_2 position, which represents carbonic anhydrase. On acid electrophoresis, a normal specimen shows a band in the A position and a small band in the F position. This band likely represents a small amount of glycated hemoglobin. Hb A_2 runs with Hb A.

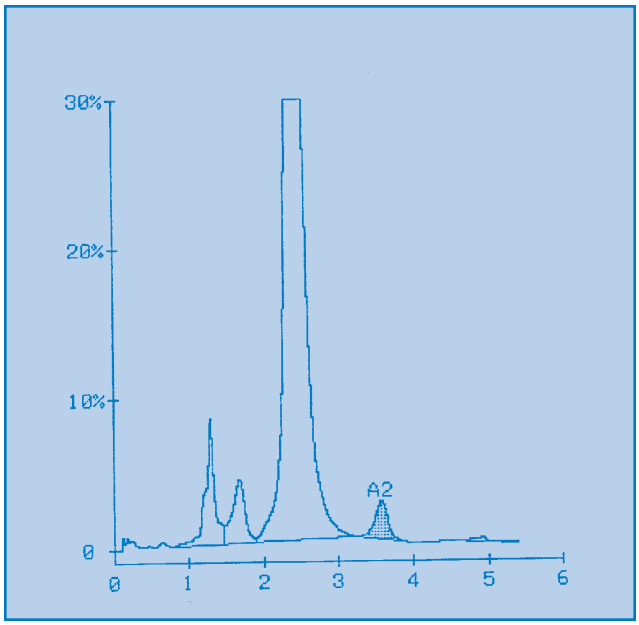


Figure 1.1

An example of a normal patient by high performance liquid chromatography. The large peak is the normal Hb A, which has a retention time of approximately 2.5 minutes. The Hb A_2 peak has a retention time of 3.3-3.9 minutes. There are two small peaks that elute before Hb A (called the P2 and P3 peaks on this instrument). These peaks usually contain glycated hemoglobin (such as Hb A_{1c}).

Case 2

HISTORY

The patient is a 26-year-old male of Southeast Asian origin who was evaluated for anemia. The physical exam was normal.

BLOOD COUNT DATA

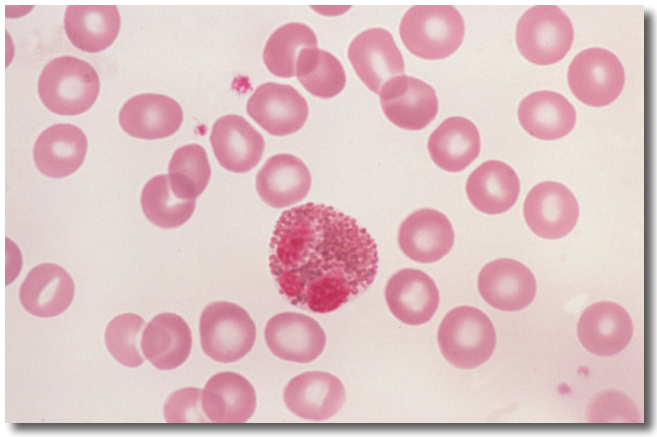
RBC.....4.1 x 10^{12}/L
Hb.....10.0 g/dL
MCV .....74 fL
WBC .....9.7 x 10^{9}/L
Plt.....234 x 10^{9}/L

PERIPHERAL BLOOD SMEAR

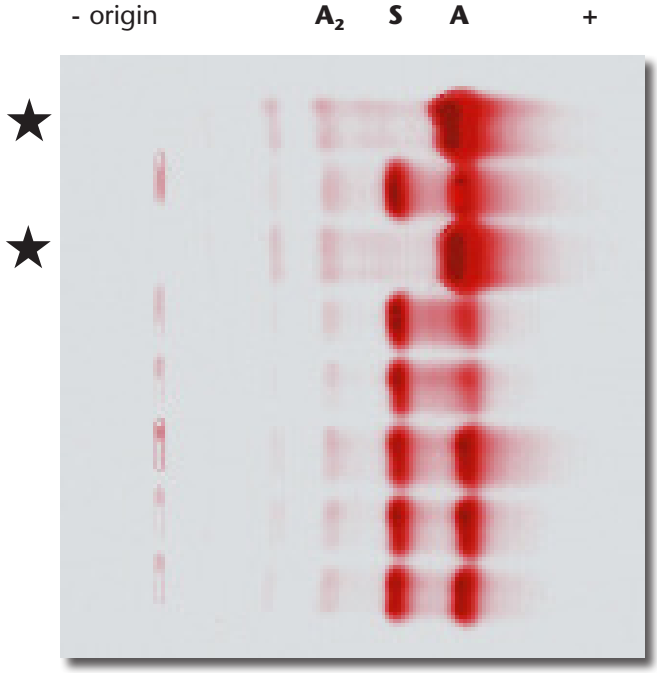
Microcytosis, hypochromia, scattered elliptocytes, and mild eosinophilia.

OTHER LABORATORY TESTS

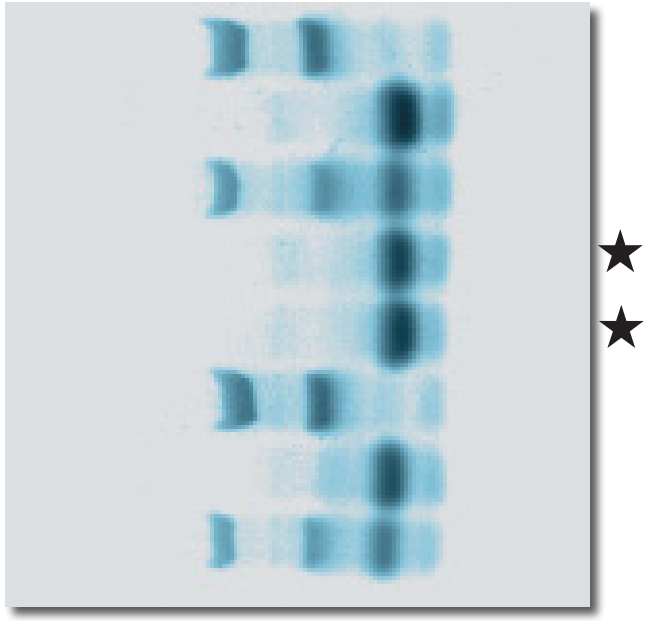
Serum iron was 86.0 µg/dL, TIBC was 277 µg/dL, and serum ferritin was 5 µg/L.



Alkaline Electrophoresis (pH 8.6)

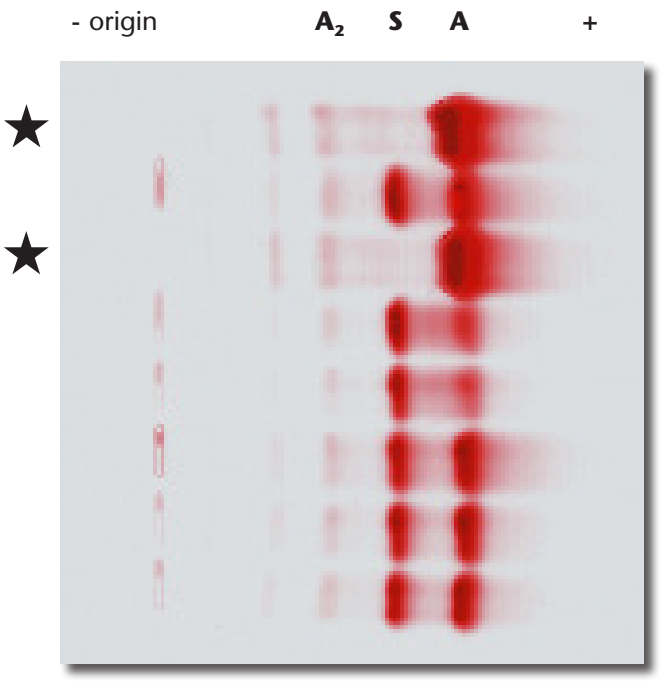


Acid Electrophoresis (pH 6.2)

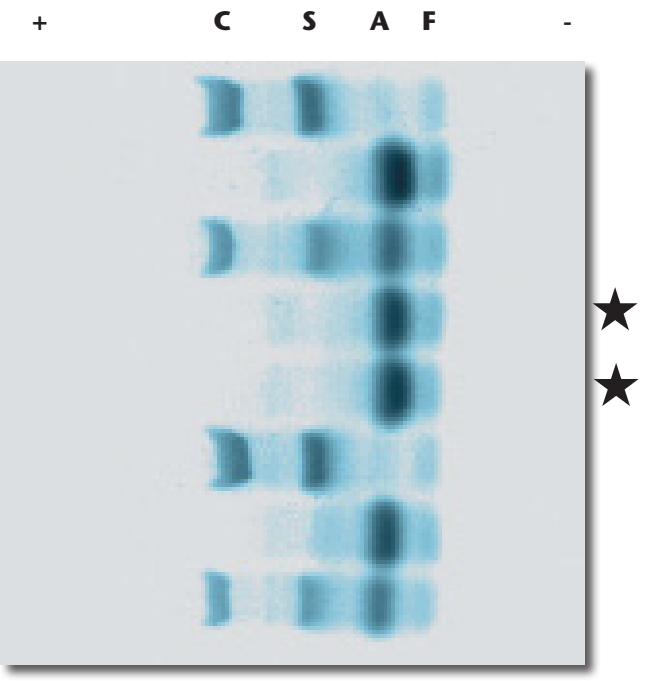


Case 2 Discussion

Alkaline Electrophoresis (pH 8.6)



Acid Electrophoresis (pH 6.2)



Interpretation

Alkaline and acid electrophoresis show a single dominant band in the A position and a normal appearing Hb A_2 band. Hb A_2 quantification revealed 2.5% Hb A_2.

Diagnosis

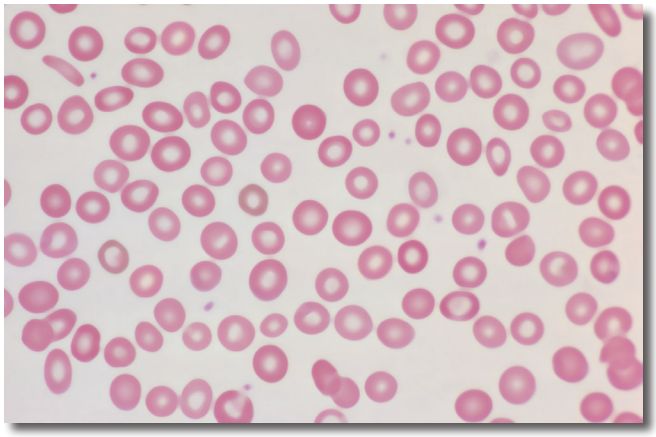
Iron deficiency anemia.

Performance

Iron deficiency anemia in a Southeast Asian patient has been used once previously in the survey. Interpretation was not required.

Discussion

The differential diagnosis in a Southeast Asian patient with a mild microcytic anemia includes the following in descending order of probability: α-thalassemia, Hb E, iron deficiency, and β-thalassemia. The low serum ferritin in this case is diagnostic of iron deficiency. It was subsequently determined that the iron deficiency was a result of chronic blood loss associated with hookworm infection, thus explaining the mild eosinophilia. However, differential diagnostic considerations in this case bear mentioning. Hb E disease is easily excluded in this case based on the absence of a major band in the C/E/O/A_2 position on alkaline electrophoresis. Heterozygous β-thalassemia (β-thalassemia minor) typically produces an elevation in Hb A_2, a finding that was absent in this case. However, it is important to point out that Hb A_2 may be in the normal range in this condition when there is concomitant iron deficiency. Thus, a re-evaluation of this patient's hemoglobin and MCV following iron repletion, and possibly a repeat Hb A_2 determination, would be necessary to completely rule out β-thalassemia in this patient. Finally, mild forms of α-thalassemia produce no electrophoretic abnormalities in adult patients, as discussed in *A Closer Look At…Alpha-Thalassemia* (page 18), and re-evaluation after iron repletion would be necessary to rule out this possibility as well. This particular patient's hematologic abnormalities completely resolved with iron therapy.



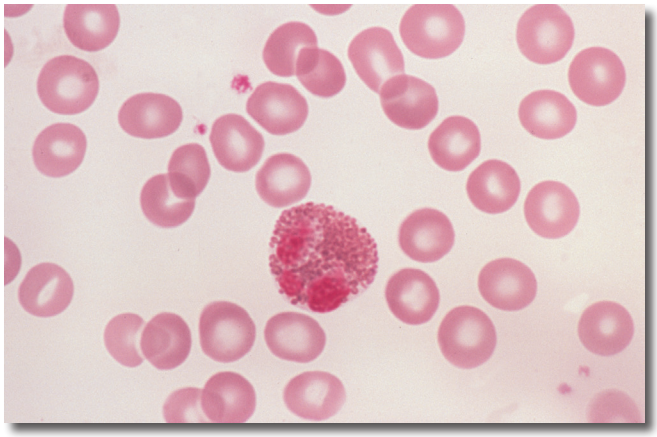


Figure 2.1 (Wright-Giemsa, 160x and 400x)
The peripheral blood smear in a patient with iron deficiency anemia. The red blood cells are hyochromic and microcytic. Central pallor is mild and there is no coarse basophilic stippling. The leukocyte is an eosinophil.

A Closer Look At...

Alpha-Thalassemia

The α globin gene cluster on chromosome 16 contains two functioning α globin genes (designated α1 and α2), as well as the embryonic ζ gene and four pseudogenes. Thus, normal individuals possess four α genes [designated (αα/αα)], with the α2 genes contributing 1.5 to 3 times more to the production of a globin chains than the α1 genes. The α-thalassemias result primarily from large deletions in the α cluster, with rare non-deletional variants. Two general haplotypes are recognized for α-thalassemia: the α-thalassemia-1 haplotype (also known α^0), in which both α genes are deleted in one α cluster [designated (--)]; and the α-thalassemia-2 haplotype (also known as α^+), in which there is a single α gene deletion in the α cluster [designated (-α)]. These two forms of mutation exist at different frequencies in different patient populations, and this becomes important from the standpoint of genetic counseling (see below). Specifically, α-thalassemia-2 trait (-α/αα) is present in 28% of African Americans, whereas the α-thalassemia-1 haplotype is virtually nonexistent in this population. In Southeast Asian, Mediterranean, and Middle Eastern populations, both types of mutation are relatively common. The α-thalassemia-1 (α^0) haplotype primarily results from a variety of deletions which abrogate both α genes on a single chromosome 16. The α-thalassemia-2 haplotype (α^+) results most commonly from a 3.7 kB deletion, which spans the 5′ end of the α1 gene, the 3′ end of the α2 gene, and intervening intronic sequences. This haplotype [designated ($-\alpha^{3.7}$), also known as the "rightward" deletion] results in a fusion α gene, with the net effect of deleting a single α gene on that chromosome. This mutation has a worldwide distribution. The second major mutation producing the α-thalassemia-2 haplotype is a 4.2 kB deletion [designated ($-\alpha^{4.2}$), also known as the "leftward" deletion] which spans and deletes only the α2 gene. This mutation is seen in Southeast Asia and Saudi Arabia but is rare in Mediterranean and black populations.

The pathophysiology of the anemia in the thalassemic disorders relates to the imbalance between the production of the α and β globin chains. When α chains are under-produced in the α-thalassemias, the excess β chains tetramerize to form Hb H. These tetramers are soluble but unstable, and thus denature and precipitate as the circulating red cells age. This leads to decreased deformability and ultimate removal by the spleen.

The clinical consequences of the α-thalassemias depend on how many α genes are deleted. The one-gene deletion [heterozygous α-thalassemia-2, (-α/αα)] is clinically and hematologically silent. The 2-gene deletions, known as α-thalassemia minor or trait [heterozygous α-thal-1 (--/αα) or homozygous α-thal-2 (-α/-α)] produce a mild, asymptomatic, microcytic anemia similar to β-thalassemia minor (Figure 2.1). (See also Case 3.) Three-gene deletions [compound heterozygous state for α-thalassemia-1 and α-thalassemia-2 (-α/--) or co-inheritance of α-thalassemia-1 and hemoglobin Constant Spring (--/α^{CS}α)] produce a moderately severe, chronic hemolytic anemia (similar to β-thalassemia intermedia) that does not require regular transfusion support. Finally, the complete absence of α genes, and thus α globin chains, produces a severe non-immune hydrops fetalis and is essentially incompatible with life (although rarely an infant may be rescued with intrauterine transfusions). The two clinically significant α-thalassemia syndromes are extremely rare in the African-American population because they both involve α-thalassemia-1 mutations. The α-thalassemias are summarized in Table 2.1 and Figure 2.2.

In regard to laboratory diagnosis, the silent carrier state is undetectable with routine laboratory studies

but may be suspected from family studies. However, the common 3.7 and 4.2 kB deletions may be easily detected with Southern blot analysis. Alpha-thalassemia minor/trait is a diagnosis of exclusion in routine practice, as it produces no electrophoretic abnormalities in adult patients. However, it may be detected at birth, as there will be a small amount of fast-moving Hb Bart's (γ tetramers) present. In addition, it may be confirmed with molecular analysis of the α gene cluster. Hemoglobin H disease is diagnosed by the presence of 20-40% Hb Bart's at birth or 5-40% fast-moving Hb H (β tetramers) in adult patients. A very small, slow moving Hb Constant Spring band may be detected as well. In addition, supravital staining with brilliant cresyl blue will produce numerous pale blue Hb H inclusions in patient red cells. Finally, 4-gene deletion hydrops fetalis is characterized electrophoretically by a predominance of Hb Bart's with smaller amounts of Hb H and Hb Portland (an embryonic hemoglobin that normally is absent by the twelfth week of gestation).

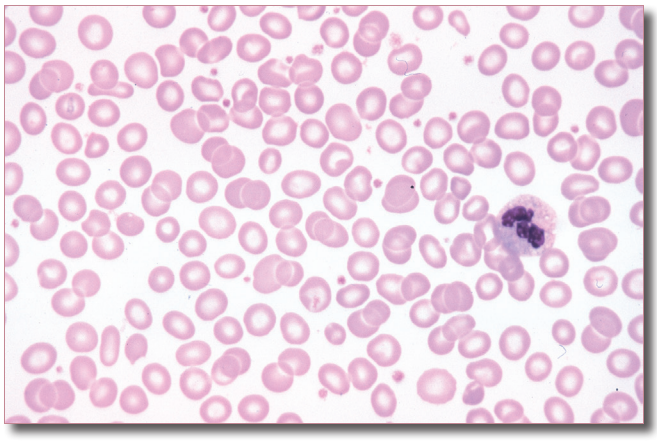


Figure 2.1 (Wright-Giemsa, 200x)
The peripheral blood smear from a patient with alpha-thalassemia due to a two alpha gene deletion. The red blood cells are mildly microcytic with slight anisopoikilocytosis.

Table 2.1 Summary of α-Thalassemias

Subtype	α Genes Deleted	Genotype	Associated Disorder	Clinical Effect
Normal	0	αα/αα	None	None
Heterozygous α-thal-2	1	-α/αα	Silent Carrier	Asympotmatic
Homozygous α-thal-2	2	-α/-α	Thalassemia minor	Microcytosis +/- mild anemia
Heterozygous α-thal-1	2	--/αα	Thalassemia minor	Microcytosis +/- mild anemia
α-thal-1/α-thal-2	3	--/-α	Hemoglobin H disease	Chronic hemolytic anemia
Homozygous α-thal-1	4	--/--	Bart's hydrops fetalis	Lethal