

Supplementary Appendix

Haemoglobin electrophoresis and HPLC

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1. Haemoglobin electrophoresis in cellulose acetate at alkaline pH (cellogel; pH=8.3 or pH=8.6). At alkaline pH, haemoglobins are negatively charged proteins so they move toward the anode (+), as shown in Figure S1.

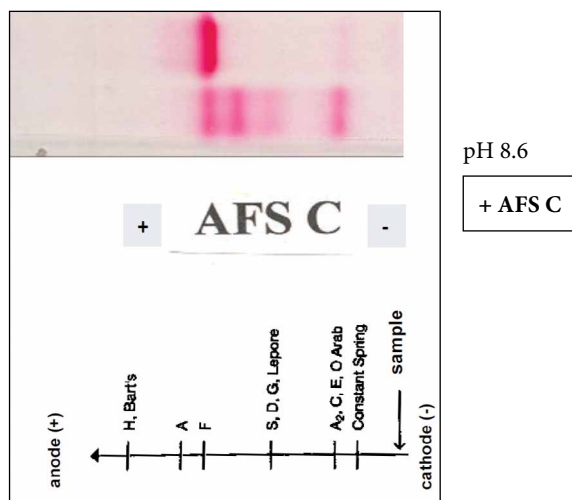


FIGURE S1. Alkaline haemoglobin electrophoresis.

2. Haemoglobin electrophoresis in agarose citrate at acid pH (agarose gel, pH=6.0 or pH=6.2 or pH=6.5). At acid pH, haemoglobins are positively charged proteins so they migrate toward the cathode (-), as shown in Figure S2.

- At alkaline pH, haemoglobins S, D, G migrate together at the same position. Hb Lepore (δ - β fusion hybrids) migrates very close to S/G/D. Their distinction is possible with electrophoresis at acid pH, HPLC and sickling test. Three Lepore haemoglobins have been identified on the basis of δ - β crossover: Lepore-Boston (also called Lepore-Washington or Hb Pylos), Lepore-Hollandia, and Lepore-Baltimore. Hb Lepore results in a β -thalassaemia-like condition: heterozygous Hb Lepore resembles thalassaemia

minor and the homozygous state results in a thalassaemia major-like condition. **Hb D** has a limited distribution (Punjab region at India-Pakistan border, where its incidence is 3%) and is clinically mild. Hb D heterozygotes are completely asymptomatic; Hb D homozygotes have mild anaemia with many target cells in the blood film or they are asymptomatic. **Hb G** is a rare α chain variant seen in Ghana and in African-Americans (Hb G^{Philadelphia}). Hb G is stable and is not associated with haematological abnormalities.

- There are 6 haemoglobins associated with the **sickling phenomenon** except Hb S (they all have the mutation $\beta 6$: Glut \rightarrow Val plus one additional point mutation): Hb C^{Harlem}, Hb C^{Georgetown}, Hb S^{Antilles}, Hb S^{Oman}, Hb S^{Travis}, and Hb S^{Providence}. They are associated with a (+) sickling test and (+) solubility test, but migrate at a different position on alkaline Hb electrophoresis and HPLC. Clinically, these haemoglobins behave as Hb S.
- **Hb I** (an α chain variant, stable, no symptoms) and a large quantity of Hb Barts ($\gamma 4$) may give a (+) solubility test. The clinical importance of Hb I is that it migrates at the same position as Hb H in alkaline electrophoresis (fast Hb variant). Hb I is not associ-

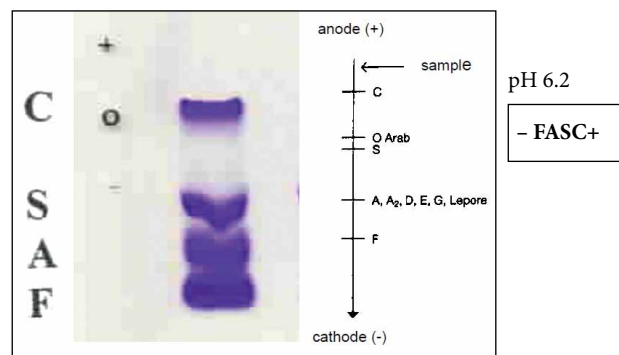


FIGURE S2. Acid haemoglobin electrophoresis.

ated with Hb H inclusions or golf-ball cells. Hb I is found in the Mediterranean littoral and in Africa.

- **Hb O^{Arab}** is rare in the tropics. Hb O is a β haemoglobin variant: Glut \rightarrow Lys (β 121). Hb O is characterised by the formation of denser and more spherical erythrocytes, leading to elevated MCHC in combination with a slight decrease in MCV. The clinical importance of this haemoglobin is that it migrates at the same position as Hb C in alkaline Hb electrophoresis, but they are separated on acid Hb electrophoresis. Haemoglobin O-Arab heterozygotes show no clinical manifestations; homozygotes present with mild haemolysis and splenomegaly of minimal clinical significance, but may develop haemolytic anaemia during infection or severe illness. Importantly, the anaemia caused by combinations of Hb O-Arab with β thalassaemia trait (β^+ or β^0) varies from benign to transfusion-dependent, and sickling is enhanced when Hb S and Hb O^{Arab} coexist. Although Hb O^{Arab} is widely distributed, it is mostly detected in Eastern Mediterranean and Middle East populations. The Greek Pomaks, a Muslim population of the mountainous area of Thrace, demonstrate Hb O^{Arab} in impressively high percentage (5.076%), which reaches 27.4% in selected villages (Hb O^{Thrace}).

3. Cation-exchange High Performance Liquid Chromatography (HPLC)

- The normal HPLC pattern is shown in Figure S3.
- Normal values: **HbA2** = 1.9-3.3% and **HbF** = 0-2%

Example 1: A 13-year-old girl of Filipino descent, with hypochromia, microcytosis, and many target cells. No history of transfusion and her parents are healthy. Figure S4 shows her HPLC. Diagnosis: Hb E heterozygote (Hb AE).

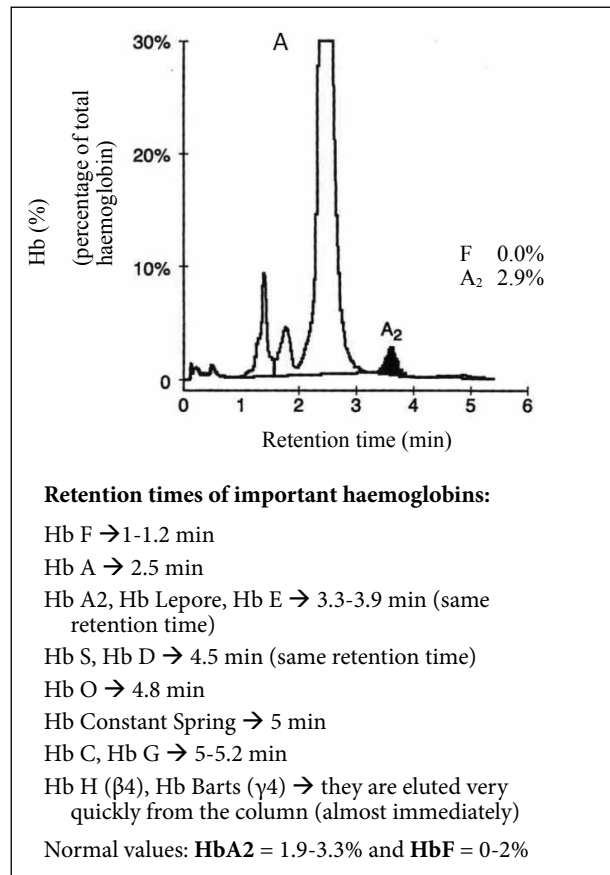


FIGURE S3. Normal HPLC pattern.

Example 2: A 33-year-old man from Nigeria with anaemia (Hb 10.0 g/dl, MCV 82 fl), splenomegaly and recurrent leg pain. No history of transfusion. His family history is unknown. Figure S5 shows his HPLC. Diagnosis: Hb SC disease. J. B. S. Haldane first suggested that that the geographi-

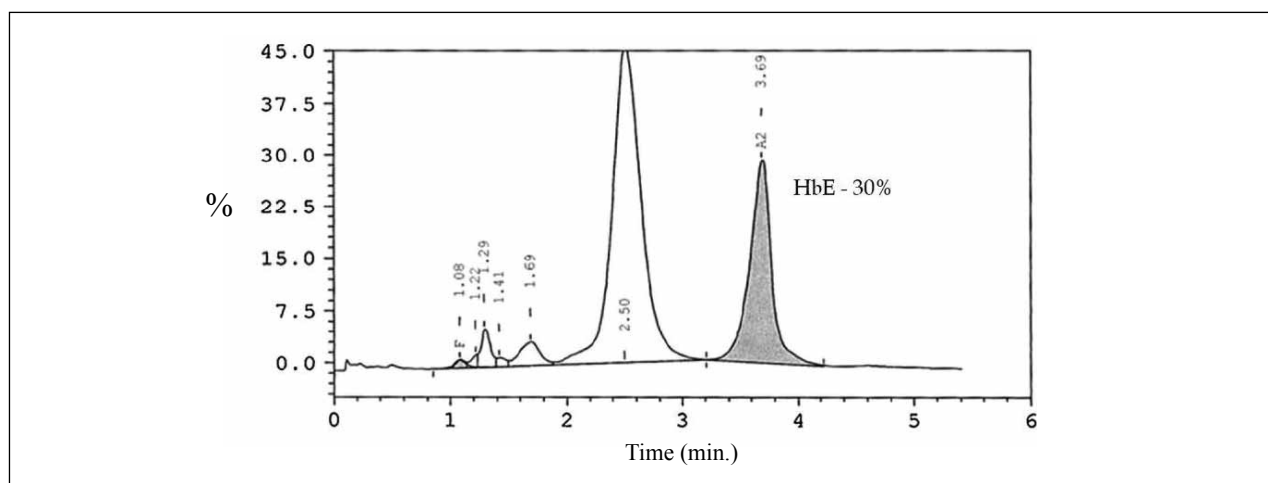


FIGURE S4. HPLC consistent with heterozygous HbE (HbAE).

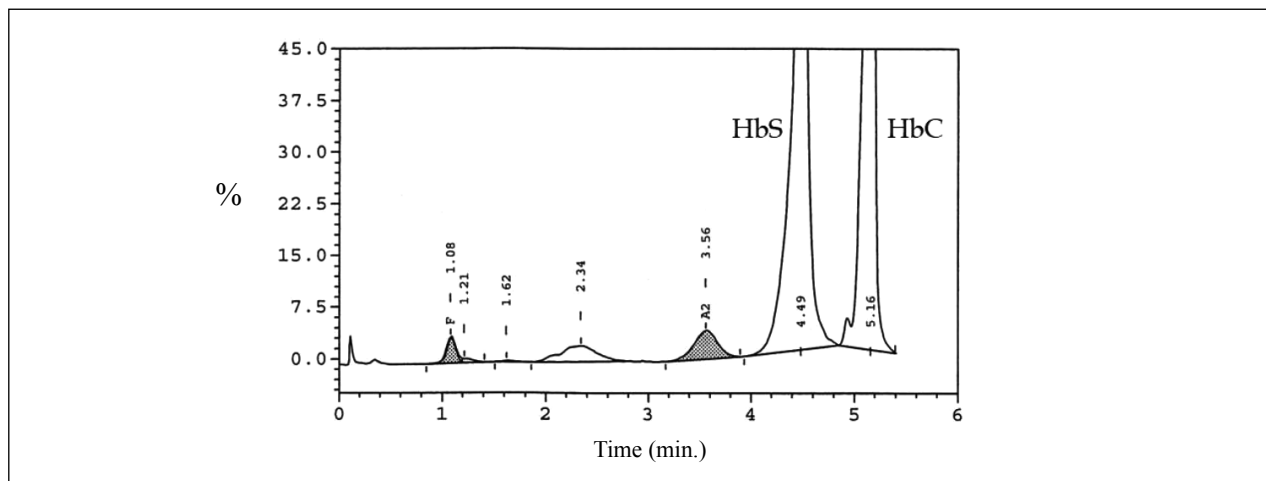


FIGURE S5. HPLC consistent with HbSC disease.

cal co-incidence of malaria and β -thalassaemia major (Cooley's anaemia) could be due to the heterozygotes (β -thalassaemia minor) being at genetic advantage through a partial protection against *P. falciparum*. A relative resistance to malaria was confirmed in Liberian children with thalassaemia minor (β/β^+). Another classic example of what Haldane called **balanced polymorphism** (i.e. heterozygotes are protected against malaria while the harmful genetic effects are restricted to homozygotes) is Hb S. African children who are heterozygous for Hb S are 10 times less likely to develop life-threatening complications of *P. falciparum* infection than those who lack this allele.

Tips:

1. Always obtain a family history when haemoglobinopathy or thalassaemia is suspected!
2. The diagnosis of heterozygous β -thalassaemia (β -thalassaemia minor) depends upon finding an increased Hb A2 >3.5%, usually 4-6% (a higher value may be seen in some cases but values of Hb A2 >7% are rare). Hb F is slightly increased in 40-50% of individuals with heterozygous β -thalassaemia (usually up to 3%; in β/β^0 trait up to 5%). In cases of:
 - HbF >5% \rightarrow consider $\delta\beta$ -thalassaemia carrier (Hb A2 <3%) or HPFH heterozygote (Hb F 5-16%).
 - low Hb A2 (<1.9%) \rightarrow consider co-inheritance of δ -thalassaemia
 - Hb A2 \geq 19% \rightarrow consider Hb E (Hb E migrates at the same position as HbA2 on alkaline and acid Hb electrophoresis and HPLC).
3. In carriers of sickle cell anaemia (Hb AS), the percentage of Hb S is usually 35-45% (because the rate of Hb S synthesis is slower than Hb A). If:
 - Hb S is <33% \rightarrow consider S- α thalassaemia co-inheritance.
 - Hb S is \geq 50% \rightarrow consider S- β thalassaemia (also has

an increased Hb A2 3.5-5% and HbF 5-10% or more) or sickle cell anaemia and recent blood transfusion.

I have found the following references of considerable value in preparing this manuscript. Many further references will be found in each of these works.

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