HAEMATOLOGY CASE STUDIES – SICKLE CELL SS

Here are a series of diagnostic case studies comparing normal neonate and adult haematology results to that of someone with sickle cell. These cases can be used as a discussion point for students studying medical or biomedical sciences.

These resources have been provided by the Leicester Royal Infirmary Department of Pathology, with special thanks going to Keith Chambers.

Blood Smear

IEF (Isoelectric focusing)

HPLC (High performance liquid chromatography)

Solubility Test

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http://www.sicklecellanaemia.org/teaching-resources/resources/scooter44-63/scooter44.html

The distinctive blood smear displaying the presence of sickle-shaped red blood cells (RBC). The RBC do not show the typical dense staining with area of central pallor, and instead are referred to as “target cells” with a visible central target and outer lighter ring.

This smear features anisocytosis meaning variation in the size of the RBC.
The IEF confirms the presence of HbS in the unknown sample (left panel) along with HbF and HbA2. The HPLC test showed a 3.5% HbA, but no HbA is present in the IEF result, suggesting that the HbA observed could have been a remnant from a recent blood transfusion. These nuances are important to understand because the presence of HbA could also have been an indication of beta thalassaemia carrier.
HPLC trace indicating sickle cell anaemia (SS).

The important features include low A0, raised A2 and a large S peak of 83%.

Although no A0 peak (for HbA) is visible on the trace, the percentage value reads 3.5%. This could be an artifact, but the results would need to be verified to be sure the 3.5% peak was A0 or not using the IEF technique.

A2 is raised beyond the 3.7% reference value to 5%, so further tests would be essential to determine whether this was SS or S with a beta thalassaemia gene. In SS, HbA2 should be within the normal range.

Another significant feature could be the presence of HbF visible on both the HPLC trace and the IEF. The clinical consequences and extent of sickling is influenced by HbF. In adult hood, HbF should be zero, but a disorder can occur called HPFH (hereditary persistence of foetal haemoglobin) in which HbF production continues into adult hood. Interestingly, high HbF is protective against sickling, and some therapeutic treatments for sickle cell include drugs to boost HbF production. The mechanisms of action are complex and more than just boosting HbF as a substitution to more serious HbS.

How does HbF work? At the molecular level, during cell sickling where the RBC undergoes a structural change losing its biconcave shape, the globin molecule that is normally wound like a ball of wool stretches into two tactoid rods. The mutation in valine causes the tactoids to bind together. HbF prevents this binding by sitting between the valine molecules and blocking the interaction and thus stopping the cells from sickling. The point to consider is that RBC are mixed bags of Hb variants, not just containing one type.
*Note of caution. The A2 peak could also be another Hb variant eluting at the same time point, and therefore increasing the percentage value. Remember, the peaks do not correspond to individual variants, but simply Hb size. Where there is any doubt over the results of a test that cannot be verified within a hospital laboratory, blood samples can undergo full DNA analysis e.g. at National Laboratory in Oxford to determine the nature of the raised A2 and whether there was a beta thalassaemia gene mutation.

All HPLC tests are verified by at least one alternative technique. The Tosoh equipment elutes HbS at around 6.01 but this doesn’t mean the peak is definitely HbS. Other variants elute at this point for example HbO Arab, although the trace will label it as HbS.

**SOLUBILITY TEST**

**TOP**

**Confirmatory Testing**

The sickle cell solubility test (Microgen Bioproduct S Tests) is a rapid procedure that identifies the presence of HbS, but it does not distinguish between combinations, i.e. AS, SS or SC.

The test is based on the solubility of Hb which varies depending on the presence of HbS. The test becomes cloudy at levels of HbS greater than 20%, but the level of cloudiness is no indicator of AS or SS since the carrier HbAS levels could be as high as 35-40%.

*Caution. There may be false readings if someone with SS has had a recent blood transfusion, so the test might appear falsely normal and clear. A holistic view of the patient history would be taken into consideration. Also in neonates where HbS is not yet formed and HbF predominates, the test will be negative.

Conclusion of confirmatory test is of a sickle cell carrier if the screening test indicates the presence HbA plus HbS in the proportions expected in an individual with beta chain variant carrier status, or no co-inherited thalassaemia.