Confirmation of HbS Haemoglobin Variant in Unknown Blood Samples

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Introduction

Haemoglobin S (HbS) ranks among the most widespread and clinically significant haemoglobin variants globally, presenting with severe and life-limiting symptoms (Thom et al., 2013). Various genotypes of this variant give rise to different forms of sickle cell disease, with the most prevalent and severe manifestation being homozygous sickle cell anemia, identified by the HbSS genotype (Rees et al., 2010). Conversely, individuals with the heterozygous sickle cell trait, denoted as HbAS, often remain asymptomatic (Kanter & Kruse-Jarres, 2013). Although the carrier status itself does not adversely affect the individual, it carries potential implications for offspring, particularly if the other parent also possesses the sickle trait, potentially predisposing the child to homozygous sickle cell anemia. The implications of sickle cell disease include life-threatening symptoms, such as painful vaso-occlusive crises and haemolytic anaemia (Osunkwo et al., 2021).

Antenatal and neonatal screening emerges as an invaluable programme for determining the risk of inheriting sickle cell disease and detecting the presence of HbS, thereby allowing for timely and effective interventions for improved outcomes and the prevention of avoidable complications, such as vaso-occlusive crises (James & Dormandy, 2019). These crises result from the rigid, sickle-shaped morphology of the mutated erythrocytes, a consequence of deoxygenated HbS polymerization (Das & Sharma, 2020).

Despite its potential to induce symptoms in those with a homozygous genotype, the sickle mutation has demonstrated some advantages for carriers. Notably, the sickle cell trait offers significant protection against malaria, primarily from *Plasmodium falciparum*. Numerous theories have been suggested to explain this protective effect, including the induction of the early breakdown of phagocytosed red blood cells, rendering them less susceptible to parasitic invasion. This phenomenon has led to a high prevalence of sickle cell mutations in regions with elevated malaria transmission, particularly in certain areas of Africa (Aneni et al., 2013).

Using a high phosphate buffer HbS can be detected due to its insolubility in its deoxygenated form causing the solution to appear turbid and cloudy (Wild & Bain, 2017). This is one method that is adopted in antenatal screening within the NHS.

Aim

The objective of this practical was to evaluate the presence of HbS within unknown samples compared to known control samples using a haemoglobin solubility method. The clinical relevance of the results was then investigated as well as the practicality of the method used.

Method

For this practical, a CLIN-TECH Sickle test kit was used as well as a positive and negative CLIN-TECH control. The test kit contains a bottle of buffered saponin and bottles of sodium dithionite. Appropriate PPE was donned prior to the practical including a laboratory coat and disposable gloves. Before commencing with the practical, the controls were removed from freezer to defrost for 20 minutes. Whilst defrosting, a bottle of sodium dithionite was reconstituted with one bottle of buffered saponin to create a reaction mixture and mixed on a roller mixer for 5 minutes. The date of the reconstitution was recorded as this is stable for 1 month and can be reused. 5 small glass tubes and the 3 unknown samples were gathered. The glass tubes were labelled – 3 with a number relating to an unknown samples, 1 with a '+' to signify the positive control, and the last with a '-' to signify the negative control as shown in Figure 1.

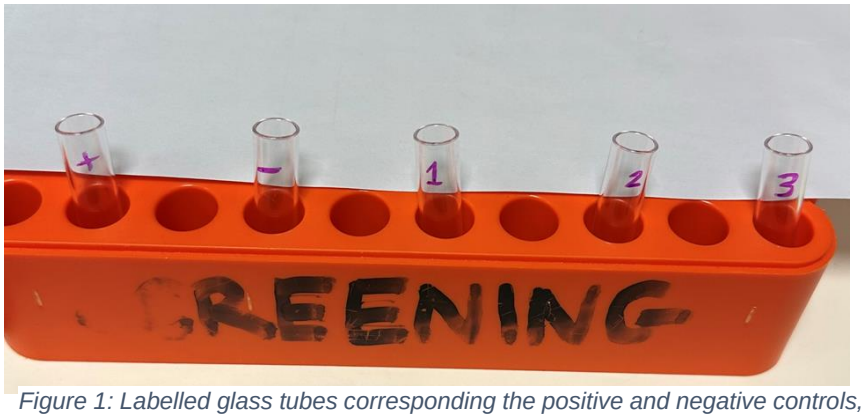

Figure 1: Labelled glass tubes corresponding the positive and negative controls, and 3 unknown samples.

Once the controls were defrosted, 1.5ml of reaction mixture was added into each tube using an automated pipette. 15ul of sample was then to the reaction mixture using an automated pipette from each sample into their corresponding glass tubes. A pastette was then used to thoroughly mix the sample and reaction mixture together ensuring no bubbles were formed. A timer was set for 3 minutes to wait for the reaction to take place. After this, the positive and negative controls were added to either end of a line-backed rack so the samples can be easily compared. The controls were then observed for changes to ensure the results were as intended. If the lines behind the rack are visible through the mixture (clear solution), this would indicate a negative result. If the lines behind the rack are not visible through the mixture (turbid solution), this would indicate a positive result. This is shown in Figure 2.

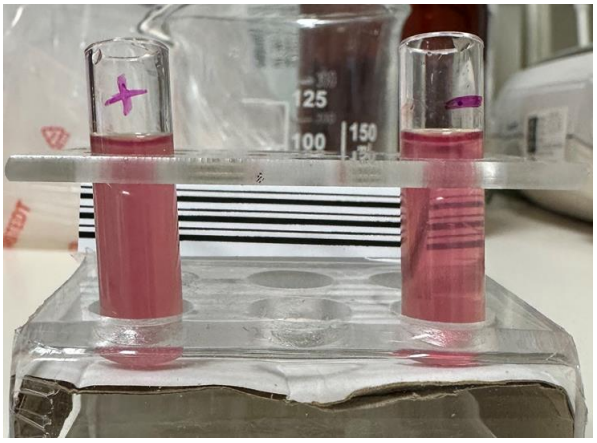

Figure 2: A line-backed rack containing a positive control on the left and a negative control on the right.

Each sample was added one at a time to the centre position of the rack to observe the results of the reaction. A senior member of staff was asked to also check the results of each sample.

Results

Table 1: A table showing the presentation of the solution for each sample following a haemoglobin solubility test and the corresponding result.

Sample	Presentation of Solution	Result
Positive Control	Turbid	Positive
Negative Control	Clear	Negative
Unknown 1	Turbid	Positive
Unknown 2	Turbid	Positive
Unknown 3	Clear	Negative

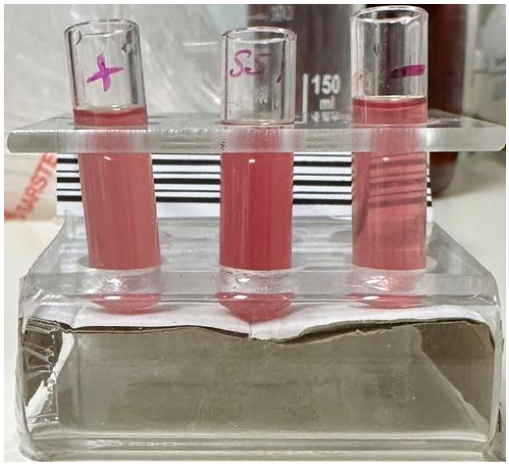

Figure 3: A photo showing the reaction solution of Sample Unknown 1 centrally in a line-backed rack with controls either side.

Figure 4: A photo showing the reaction solution of Sample Unknown 2 centrally in a line-backed rack with controls either side.

Figure 5: A photo showing the reaction solution of Sample Unknown 3 centrally in a line-backed rack with controls either side.

Discussion

The principle of sickle solubility test is based on the insolubility of HbS. When saponin is mixed with a whole blood sample, it haemolyses it. Adding sodium dithionite then reduces the haemoglobin, however, in the presence of a concentrated phosphate buffer, which in this case is supplied in the buffered saponin reagent, HbS is insoluble. This means that if there is HbS present in the whole blood sample the solution would turn turbid, however, if there is no HbS present then the haemoglobin should remain in the solution since it is soluble. This leads to the solution remaining clear and transparent (Mohanty et al., 2021). Using this knowledge, the results from this practical can be assessed.

Table 1 shows that both the controls have resulted as expected with the positive control being turbid, indicating the presence of HbS, and the negative control being clear, indicating the absence of HbS. This suggests that the reactions have been successful and the results for the unknown samples are reliable. Table 1 also shows that unknown samples 1 and 2 were positive for the presence of HbS due to the turbid solution, and unknown sample 3 was negative for HbS due to the solution being clear.

In many cases in the UK, a sickle solubility test is only used as confirmatory test following detection of sickle cell using HPLC analysis (NHS, 2017). If this were the case with these unknown samples then the negative solubility test result should be investigated further to understand why the HPLC analysis showed the presence of HbS. The sickle solubility test has shown to suffer from false-negative results in a variety of cases. For example, false negatives are likely when the percentage of HbS in the whole blood sample is <10%. This can affect patients who suffer from severe anaemia as well as newborns who have a higher prevalence of foetal haemoglobin. As well as this, coinciding blood disorders such as α-thalassemia can interfere with the results and cause a false negative (Arishi et al., 2021).

The sickle solubility test is highly specific and sensitive to the presence of HbS however it is unable to determine the presence of other haemoglobin variants. It is also unable to distinguish the genotype. Therefore, the type of condition the patient has cannot be differentiated. For example, they may be a homozygous HbSS carrier and suffer from sickle cell disease but alternatively they may be a carrier of the sickle cell trait and have the HbAS genotype. Due to this limitation of the test, in a clinical setting, other methods of testing may be preferred that offer a weirder range of variant identification such as haemoglobin electrophoresis, isoelectric focusing, or HPLC to get a better understanding of the patient's genotype. This could impact ay treatment they require and, in the case of antenatal screening, impact the decision for further testing and partner screening (Tubman & Field, 2015).

Conclusion

The sickle solubility test plays an integral role in the testing and confirmation of the presence of sickle cell haemoglobin. It is a cheap and fast test that can be integrated into point of care testing which can be beneficial in areas of high sickle cell prevalence (Arishi et al., 2021). Despite the benefits of this test, it is not often the first test used in the clinical setting as it limits the identification of haemoglobin variants to only HbS whereas there are many other methods, such as HPLC, which identify HbS as well as a large panel of other haemoglobin variants which can be beneficial in the diagnosis of patients.

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