MBP003

Hi-Speed Sickle Kit
(Centrifugation based detection of Hemoglobin ‘S’)

Kit Contents

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Reagents / Materials provided</th>
<th>MBP003</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS0081</td>
<td>Reagent S</td>
<td>115 ml</td>
</tr>
</tbody>
</table>

Empty Reaction Tubes will be provided with the Kit

Introduction

Human hemoglobin is formed from two pairs of globin chains each with a heme group attached. The binding of a heme group into the heme pocket in each chain is vital for the oxygen-carrying capacity of the molecule and stabilizes the whole molecule. Alterations in the structure of hemoglobin are usually brought about by point mutations that affect the coding for amino acids in the globin chains.

In sickle cell anemia, a point mutation (GAG to GTG) in the β-chain at codon position 6 results in the encoding of a valine instead of normal glutamine. The resulting abnormal β-chains combine with normal β-chains to form abnormal hemoglobin S (HbS). HbS is poorly soluble in low oxygen tension situations forming a gel and polymerizing into fibrilary structures or tactoids. This distorts the red blood cells causing them to become rigid and sickled.

HbA Normal Hemoglobin
HbAS Sickle cell trait
HbS Sickle cell anemia

Individuals with sickle cell anemia (Homozygous S/S) may have early mortality with vascular occlusions of multiple organ system, severe hemolytic anemia and hypoxia. Individuals with sickle cell trait (Heterozygous A/S) are usually asymptomatic. However, under certain conditions of reduced oxygen tension such as hypoxia during anesthesia, flight in poorly pressurized airplanes, severe pneumonia, these individuals can experience a sickle cell crisis.

Hi-Speed Sickle Kit

This kit is based on the solubility difference between HbS and HbA in Solubility Test Reagent. When red cells are introduced into such a solution, they lyse immediately. The hemoglobin released from the lysed red cells, is reduced by components in Reagent S provided with the kit. This reaction causes precipitation of HbS leading to turbidity of the reaction mixture. However, HbA, as well as other hemoglobins are soluble leading to clarity in the reaction mixture.

This test is simple and stable screening test and so the samples tested positive should be confirmed by electrophoresis so as to reduce the chances of False Positives.

Precautions while handling reagents

1. Reagent for laboratory use only.
2. Do not pipette by mouth.
3. The reagent can be damaged due to microbial contamination or on exposure to extreme temperature.
4. Use reagent of same lot numbers. Do not interchange reagent of different lot numbers.
Quality Control
Each test should be performed with a known positive and negative blood sample.

Storage
Store Reagent S at 2-8°C. Avoid direct exposure to sunlight. The reagents in the solubility kit have a shelf life of one 1 year (if stored at mentioned conditions).

Centrifugation
All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. The tubes provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are performed at room temperature and are given in g, the correct rpm can be calculated using the formula:

\[ \text{RPM} = \sqrt{\frac{\text{RCF}}{1.118 \times 10^{-5}}} \times \text{r} \]

where \( RCF \) = required gravitational acceleration (relative centrifugal force in units of g); \( r \) = radius of the rotor in cm; and \( \text{RPM} \) = the number of revolutions per minute required to achieve the necessary g-force.

Procedure
1. Dispense 2 ml of Reagent S with the help of a Pipette in each of the empty reaction tubes (provided).
2. Gently add 25µl of freshly collected whole blood sample to each reaction tube with the help of a Pipette.
   
   NOTE: Anticoagulated blood should be used if the test is not performed on freshly collected blood sample.
3. Gently mix the tubes for 10-15 seconds.
4. Allow the tubes to stand for 10 minutes at room temperature.
5. Centrifuge the reaction tubes at 2,500-3,000 rpm in a tabletop centrifuge for 10 minutes at room temperature (15-25°C).
6. Allow centrifuge to stop without braking and carefully remove the reaction tubes without disturbing the contents.

INTERPRETATION OF RESULTS

<table>
<thead>
<tr>
<th>Type of hemoglobin</th>
<th>Lower layer</th>
<th>Upper layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb-A (Normal)</td>
<td>Clear and dark red in color</td>
<td>Grey precipitate</td>
</tr>
<tr>
<td>Hb-AS (Sickle Cell Trait)</td>
<td>Clear and light red to pink in color</td>
<td>Red precipitate</td>
</tr>
<tr>
<td>Hb-S (Sickle Cell Anemia)</td>
<td>Clear and colourless</td>
<td>Red precipitate</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
NOTE: This Figure represents single set of experiment. Some slight color variation might be seen.

Remarks
1. Negative control samples can be collected from normal, healthy individuals.
2. All positive results should be confirmed by running agarose gel electrophoresis (MBP001).
3. This test does not discriminate between different dysgobulnemias, β-thalassemia and hemoglobin C disease.
4. The results of the test should be correlated with clinical findings to arrive at the final diagnosis.

Limitations of the test
1. Conditions like severe anemia (hemoglobin level less than 7 gm/dL) can result in false negatives.
2. Foetal hemoglobin more than 25% can result in false negative results.

References
1. A rapid whole blood solubility test to differentiate the sickle-cell trait from sickle-cell anaemia R. G. HUNTSMAN, G. P. T. BARCLAY, D. M. CANNING, AND G. I. YAWSON.

Technical Assistance
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