MBP003

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

Kit Contents

Product Code	Reagents / Materials provided	MBP003 For 50 Samples
DS0081	Reagent S	115 ml

Empty Reaction Tubes will be provided with the Kit

Intended Use

Recommended for identification of normal and sickle cell anemia human blood samples.

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 ß- chain at codon position 6 results in the encoding of a valine instead of normal glutamine. The resulting abnormal ß- chains combine with normal ß- 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.

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CE
Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800
Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

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.

Materials needed but not provided

- Centrifuge machine (with 2ml rotor and upto 10,000rpm speed)
- Pipettes [1000µl (Product Code: LA614), 200µl (Product Code: LA613), 50µl (Product Code: LA612)and 10µl (Product Code: LA611) capacity]

Storage

Store Reagent S at 2-8^{0}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:

$$RPM = \sqrt{RCF/1.118 \times 10^{-5} r}$$

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

Specimen Handling and Collection

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future). Ensure that the blood sample is at room temperature before beginning the protocol.

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.

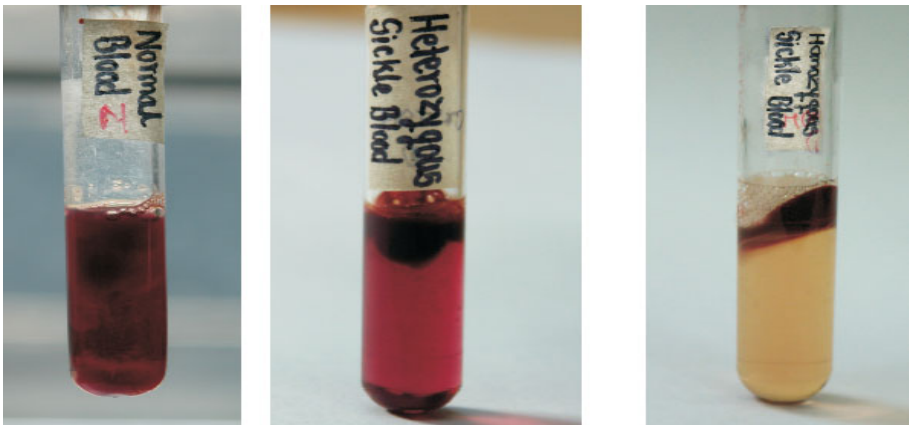
4. Allow the tubes to stand for 10 minutes at room temperature.

5. Centrifuge the reaction tubes at 2,500-3,000 rpm in a tabletop centrifuge for 10 minutes at room temperature (15-25^{O}C).

6. Allow centrifuge to stop without braking and carefully remove the reaction tubes without disturbing the contents.

INTERPRETATION OF RESULTS

Type of hemoglobin	Lower layer	Upper layer
Hb-A (Normal)	Clear and dark red in color	Grey precipitate
Hb-AS (Sickle Cell Trait)	Clear and light red to pink in color	Red precipitate
Hb-S (Sickle Cell Anemia)	Clear and colourless	Red precipitate



NORMAL BLOOD **HETEROZYGOUS BLOOD** **HOMOZYGOUS BLOOD**

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 or MBP008).
3. This test does not discriminate between different dysglobulnemias, 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.

Warning

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

Each lot of HiMedia's Hi- Speed Sickle Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	Turbidity observed	Clarity
Normal blood sample	No	Yes
Sickle blood sample	Yes	No

I. Negative- If solution is clear and black lines visible.
II. Positive- If Solution is turbid and black lines not visible.

NOTE: Each test should be performed with known positive and negative control blood samples

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.
2. Practical Haematology , Sir John Dacie, Ninth edition, 2001.
3. Clinical Diagnosis and Management , J.B Henry , 20th Edition , 2001.

Safety Information

Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

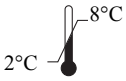
Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Please refer disclaimer Overleaf.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, send an email to mb@himedialabs.com.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
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	HiMedia Laboratories Private Limited, Reg. Off: Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate, Thane, (West) 400604, Maharashtra, INDIA. Web: www.himedialabs.com
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu
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