

### Rapid Test for Sickle Cell Anemia – Device

(Rapid immunochromatographic assay for simultaneous detection and differentiation of Sickle Hemoglobin (HbS) and Normal Hemoglobin (HbA) from human whole blood)

#### For In-Vitro Diagnostic Use Only

**Store at 2°C to 40°C**

#### INTRODUCTION

Sickle Cell Anemia is a group of inherited disorders that affect hemoglobin, the major protein that carries oxygen in red blood cells. Normally, red blood cells are disc-shaped and flexible so they can move easily through the blood vessels. In sickle cell disease, red blood cells are typically "sickle"-shaped due to a gene mutation. Sickle Hemoglobin (HbS) which is different from the normal Hemoglobin (HbA). They differ at the 6th amino acid position, where HbA has glutamic acid, and HbS has valine. These sickle-shaped cells cause blockages in the blood flow.

#### INTENDED USE

The product is intended for Simultaneous detection and differentiation of Sickle Hemoglobin (HbS) and Normal Hemoglobin (HbA) from human whole blood.

The product is for professional IVD use only.

#### PRINCIPLE

After addition of the diluted whole blood sample to the sample well of the device containing a test strip, the sample moves on to the conjugate pad containing colloidal gold particles conjugated with PAN Hb specific Antibodies and Rabbit IgG. If the sample contains detectable levels of the Sickle Cell Anemia (HbS) specific Hb, it reacts with the gold conjugated PAN Hb Specific Antibodies to form a complex. This complex moves further and reacts with Anti-HbS Antibody coated as a separate test line on the nitrocellulose membrane to form colored band. The normal Hb (HbA) also binds to gold conjugated PAN Hb Specific Antibodies to form a complex. This complex moves further and reacts with Anti-HbA Antibody coated as a separate test line on the nitrocellulose membrane to form colored band. The formation of colored line at HbA denotes presence of normal Hb. While, the formation of colored line at HbS denotes presence of Sickle Cell Anemia Hb (HbS).

The unbound complex & Rabbit IgG conjugated colloidal gold particles move further to the goat anti-Rabbit IgG coated control area to form a colored band (Control line).

Appearance of only control line indicates a negative result. The control line acts as a procedural control. Control line should always appear if the test is performed as per the procedure and reagents are working properly.

#### CONTENTS OF KIT

1. Test Device with desiccant and plastic dropper in individual pouch
2. Sample Extraction tube (prefilled with dilution Buffer)
3. Plastic dropper for Blood sample collection
4. Plastic dropper for Adding diluted sample into device
5. Package Insert

#### OPTIONAL MATERIAL REQUIRED

1. Timer
2. Blood Sample container/collection tube
3. Micro pipette
4. Lancet
5. Alcohol swab
6. Disposable Gloves

#### PRECAUTIONS/KIT STORAGE AND STABILITY

1. Please read all the information in this package insert before performing the test. Pay particular attention to the position of the Control and Test lines.
2. Do not use the product after expiry date, the expiry date is printed on the foil pouch and packing of product.

3. Store in the sealed pouch in a dry place in between temperature 2°C to 30°C. Do not freeze.

4. Do not use if pouch is torn or damaged.

5. Do not open the foil pouch until you are ready to start the test.

6. Keep out of the reach of children.

#### WARNINGS

1. Do not reuse the test device.
2. Follow the instruction to get accurate results.
3. Use appropriate personal protective equipment.
4. Dispose the used test components hygienically in Biohazard waste.
5. Do not touch the membrane.
6. Treat samples and used test as potentially infectious. Avoid contact with skin.
7. For in vitro diagnostic use. Not to be taken internally.
8. Do not eat the desiccant in the package.
9. Do not mix the specimen sample or interchange the different specimen.

#### SAMPLE COLLECTION AND PREPARATION:

1. Collect finger prick fresh blood or EDTA fresh blood for testing.
2. Blood collected in EDTA tube can be used within 3 days of collection if stored 2 to 8 °C till testing or during transportation.
3. Aspirate fresh finger prick /EDTA fresh blood sample using 10 µl dropper provided with the kit (Plastic dropper for Blood sample collection).
4. Add 1 drop of whole blood (10µl) in to the sample preparation tube containing dilution buffer.
5. Mix and use this diluted sample for testing as per given test procedure.
6. Do not keep this diluted sample at room temperature for prolonged period.
7. Use the diluted sample immediately or use with in 3 hours provided the tube is capped and stored at room temperature or 2 to 8 °C.

#### TEST PROCEDURE

1. Dilute the sample as per sample preparation procedure.
2. Allow the test device and sample reach to room temperature (20°C to 30°C) before opening the foil pouch.
3. Remove the test device, desiccant and plastic dropper from the pouch and use it as early as possible.
4. Put the device on plain surface and add 3 drops (Approx. 70 - 80 µl) of diluted blood sample in sample well using the provided Plastic dropper for Adding diluted sample into device
5. Start the timer.
6. Read the result at 15 minutes. Do not read the result after 20 minutes.

#### RESULT INTERPRETATION

C: Control line  
A: HbA line (HA)  
S: HbS line (HS)

**Negative for Sickle cell disease (Presence of normal Hemoglobin HbA):** Colored line observed at control region C and HbA region (A) and Absence of colored line HbS region (S).



**Positive for Sickle cell disease (Presence of Hemoglobin HbS):** Colored line observed at control region C and HbS region (A) and Absence of colored line HbA region (S).



**Positive for both Hemoglobin HbS and Hemoglobin HbA:** Colored line observed at control region C and HbS region (S) and HbA region (A). Presence of faint clearly visible line at respective region denotes presence of traces of respective analyte.



HbAS Positive ( AS Trait)

Faint in HbA + positive in HbS: Presence of low HbA levels along with Sickle cell Disease

#### Positive for other hemoglobinopathies or thalassemia:

Colored line observed at control region C and absence of colored line at HbS region (S) and HbA region (A).



Other Hemoglobinopathies

#### Invalid:

Colored line absents at control region C and in either presence or absence of colored line at HbS region (S) or/and HbA region (S).



#### LIMITATIONS

- As with all diagnostic tests, the test result must always be correlated with clinical findings.
- The results of test are to be interpreted within the epidemiological, clinical and therapeutic context.
- Any modification to the above procedure and / or use of other reagents will invalidate the test procedure.

#### PERFORMANCE CHARACTERISTICS

##### INTERNAL EVALUATION

Total 185 samples were evaluated for specificity & sensitivity. sensitivity was found to be 100% (60/60) and relative specificity was found 100% (125/125).

The Positive predictive value (PPV) and Negative Predictive value (NPV) for the test was 100 %.

No cross reactivity found with RF, Pregnancy, High Hemoglobin, Malaria, SLE, ANA, Psoriasis, HIV positive samples.

Sample	SCA Test		Reference		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative	Positive	Negative				
Positive	60	-	60	-	100	-	100	-
Negative	-	100	-	100	-	100	-	100
Cross reactivity	-	25	-	25	No cross reactivity observed			-
Total	60	125	60	125	-			

#### EXTERNAL EVALUATION

The product is evaluated by ICMR-NIIH, government of India. 100 % sensitivity and 100 % specificity were found in external evaluation.

#### REFERENCES

- The spatial epidemiology of sickle cell anemia in India. Carinna Hockham et al. Scientific reports (2018) 8:17685.
- Pathophysiological insights in sickle cell disease. Odievre, Met al. IJMR 134, 532-537 (2011).
- Sickle cell Trait: A Benign state? Taiwo R. Kotila. Acta Haematol 2016; 136:147-151.

- Hematological and clinical features of sickle cell diseases: A Review, Donald R. Harkness. Hemoglobin, 4(3&4), 313- 334(1980).
- Sickle Cell Anemia Hemoglobin: The Molecular Biology of the First "Molecular Disease"- The Crucial Importance of Serendipity. Vernon M. Ingram. Genetics 167: 1-7 (May 2001).
- Human Hemoglobin: HUEHNS. E. R. J. med. Genet. (1965). 2, 48.
- Data on file: Zephyr Biomedicals.
- A Global Perspective on Sickle Cell Disease. Pediatric Blood Cancer. Aygun B, Odame I. 2012;56:386-90.
- The management of sickle cell disease. Adams RJ et al. Natl Institutes Heal. 2002; 1-206.
- Diagnosis of Sickle Cell Disease and Its Related Hemoglobin Disorders. Yang Y-M, Brigham S, Liu PI. In: Mankad VN, Moore RB, editors.
- Sickle Cell Disease: Pathophysiology, Diagnosis, and Management. Westport: Praeger; 1992. p. 201-20.

<b>IVD</b>	In Vitro Diagnostic Use
	Manufacturer
	Manufacturing Date
	Expiry Date
<b>LOT</b>	Lot Number
	Store at 2°C to 40°C
	Single Use
	Number of tests in the pack
	Do not use if pouch or kit damaged
	This side Up
	Read package insert before use



#### MANUFACTURED BY

ImmunoScience India Private Limited,  
Gat No. 41, Kusgaon, Shivapur-Velhe Road,  
Tal- Bhor, Pune, Maharashtra (India) – 412205.