SICKLECHECK™

Rapid test for simultaneous detection of Hb S and Hb A in human whole blood.

DEVICE

INTENDED USE

SICKLECHECK™ is a rapid, qualitative, immunochromatographic assay for the simultaneous detection of Hb S and Hb A in human whole blood sample for diagnosis of sickle cell disorder.

SUMMARY

Hemoglobin S (Hb S) differs from the normal Hemoglobin A (Hb A) by a single amino acid mutation at position 6 of the beta chain; wherein glutamic acid is replaced by valine. During low oxygen conditions, the red blood cell morphology may range from mild elongation to irreversible elongated tactoid. This elongated filamentous tactoid formation results in the typical 'sickle' appearance of the red blood cell.

Individual with sickle cell anemia (homozygous S/S) may have early mortality with vascular occlusions of multiple organ systems, severe hemolytic anemia and hypoxia. Individual 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, sever pneumonia; they can experience a sickle cell crisis.

SICKLECHECK™ is a rapid, competitive lateral flow immunochromatographic assay for the qualitative detection of Hb S and Hb A in human whole blood sample.

PRINCIPLE

SICKLECHECK™ is based on the principle of agglutination of antibodies/antisera with respective antigen in a competitive immune-chromatography format along with use of nano gold particles as agglutination revealing agent. The conjugate pad is impregnated with two components – monoclonal antibody for Hemoglobin S (Hb S) conjugated to colloidal gold, monoclonal antibody for Hemoglobin A (Hb A) conjugated to colloidal gold.

As the test specimen flows through the membrane assembly of the device, the highly specific monoclonal antibody for Hb S & Hb A – colloidal gold conjugate complexes with the respective antigens Hb S & Hb A present in the test specimen and travels on the membrane due to capillary action. The complex moves further on the membrane to the test region (S & A) where it is not captured by Hb S & Hb A coated on the test membrane, therefore forming no band. The absence of colour bands at the test regions (S & A) indicates the presence of respective antigen (Hb S / Hb A) in the test specimen.

In absence of Hb S & Hb A, the highly specific monoclonal antibody for Hb S & Hb A – colloidal gold conjugate travels on the membrane due to capillary action and moves further on the membrane to the test region where it is immobilized by specific antigen Hb S & Hb A coated on the test membrane (S & A), therefore forming colored bands. The presence of colored band at the test regions (S & A) indicate the absence of the respective antigens (Hb S / Hb A) in the specimen. The intensity of the test line is dependent on the concentration of the analyte present in the test specimen. The unbound colloidal gold conjugates moves further on the membrane and immobilized by the agglutinating sera for goat anti mouse IgG coated on the membrane at the control region (C). This control band acts as a procedural control and serves to validate the test results.

REAGENTS AND MATERIALS SUPPLIED

SICKLECHECK™ kit contains:

A. Individual pouches, each containing:
   1. DEVICE : Membrane test assembly impregnated with colloidal gold conjugated to Anti-Hb S, Anti-Hb A antibody, Hb S & Hb A and goat anti mouse IgG at the respective regions.
   2. Desiccant pouch.

B. PIPETTE : Disposable Plastic Specimen Transfer Devices with **10 µl** mark .

C. BUF : Assay Buffer vials.

D. Nozzle caps.

E. Package insert.

F. Alcohol swabs – 70% Isopropyl alcohol (optional*).

G. Sterile lancets (optional*).

H. Patient Test Data Sheet Cards (optional*).

* Optional material provided on request.

REF	508010025
₹	25 Tests

OPTIONAL MATERIALS REQUIRED

10µl calibrated micropipettes, micropipette tips, sterile safety lancets, alcohol swabs, stopwatch.

STORAGE AND STABILITY

The sealed pouches in the test kit and the kit components may be stored between 4°C to 40°C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE.

NOTES

1. Read the instructions carefully before preforming the test.
2. For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
3. Do not use the kit beyond expiry date and do not re-use the test device.
4. Do not intermix reagents from different lots.
5. Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to minimum. Inhalation / swallowing may cause harm.
6. Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
7. If desiccant colour at the point of opening the pouch has turned from blue to pink or colourless, another test device must be run.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is necessary prior to specimen collection by approved techniques.

a. Venous blood:

Collect whole blood in EDTA, Heparin, Sodium Citrate or ACD anticoagulant. Though fresh blood specimen is preferable; the specimen can be stored at 2°C to 8°C for up to 24 hours, in case of delay in testing.

Use the Specimen Transfer Device (provided in the kit) to collect blood specimen for testing. Touch the open end of the Specimen Transfer device to the blood specimen. Press the bulb of a Specimen Transfer Device and release the bulb to allow aspiration of the collected whole blood specimen upto **10 µl** mark on the Specimen Transfer Device (Refer **Step 4** of the pictorial procedure). Transfer the collected specimen into the **buffer vial** after removing its red coloured cap and the rubber plug. **Discard the rubber plug and the red coloured cap**. Cap the buffer vial with nozzle cap. Mix thoroughly by gently shaking the vial sideways, keeping the buffer vial facing upward. The specimen is now ready for testing. If immediate testing is not possible, then the specimen in the buffer vial can be stored for up to 4 hours at room temperature or 2°C to 8°C, but it must be well mixed before testing.

b. Capillary blood by finger prick:

Prick the finger (preferably ring finger of non-writing hand) with the help of lancet. Press the finger (if required) till blood oozes out freely. Wipe off the initial blood drop. Press the bulb of a Specimen Transfer Device and release the bulb to allow aspiration of the collected whole blood specimen upto **10 µl** mark on the Specimen Transfer Device (Refer **Step 4** of the pictorial procedure). Transfer the collected specimen into the **buffer vial** after removing the red coloured cap and the rubber plug. **Discard the rubber plug and the red coloured cap**. Cap the buffer vial with nozzle cap. Mix thoroughly by gently shaking the vial sideways keeping the buffer vial facing upward. The specimen is now ready for testing. If immediate testing is not possible, then the specimen in the buffer vial can be stored for up to 4 hours at room temperature or 2°C to 8°C, but it must be well mixed before testing.

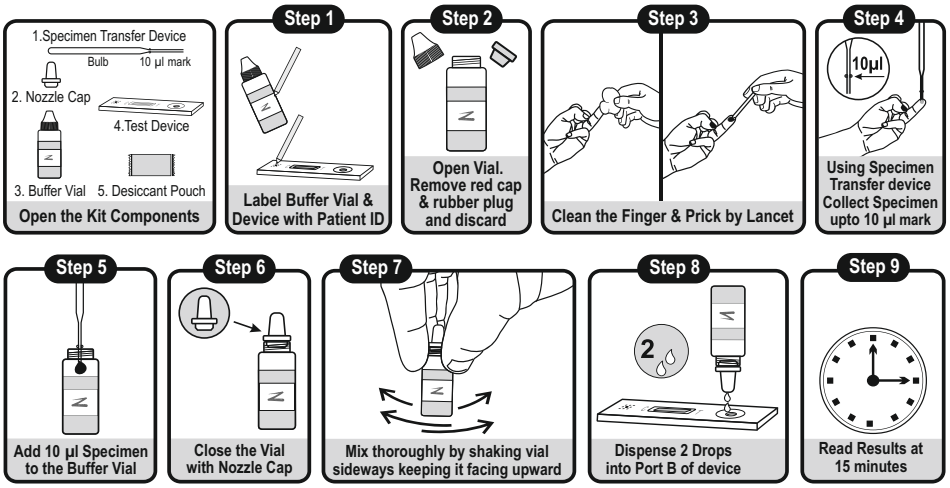
Note: Refer Pictorial Procedure as a reference.

TESTING PROCEDURE

1. Bring the kit components of **SICKLECHECK™** Device to room temperature prior to testing.
2. Open a foil pouch by tearing along the "notch" and remove the testing device.
3. Check the colour of the desiccant pouch. It should be blue. If the desiccant has turned colorless or pink, discard the test device and use another device. **Once opened, the device must be used immediately.**
4. Label the device and Buffer vial with patient/specimen identity.
5. Place the testing device on the flat horizontal surface.
6. Remove the red coloured cap and the rubber plug of the buffer vial. **Discard the rubber plug and the red coloured cap.**
7. Collect **10 µl venous blood or finger prick blood specimen** using the Specimen Transfer device.
8. Press the bulb of a Specimen Transfer Device and release the bulb to allow aspiration of the collected whole blood specimen upto **10 µl** mark on the Specimen Transfer Device (Refer **Step 4** of the pictorial procedure).
9. Transfer the collected specimen into the labelled Buffer vial.
10. Cap the Buffer vial with the nozzle cap.
11. Mix the contents of the labeled buffer vial by gently shaking the vial sideways, keeping the vial facing upward.
12. Invert the vial and carefully dispense exactly **two drops** of specimen-buffer mixture into the specimen port (B) of the test device, holding the vial in vertical position.
13. Observe the development of visible colored band at test regions (S & A).
14. Visible bands may be observed at the end of **15 minutes**, depending on the concentration of the analyte present in the specimen
15. Do not read and interpret after 15 minutes.

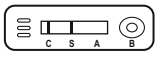
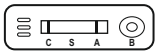
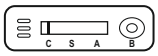
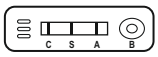
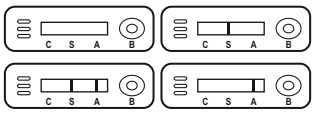
Note: Each patient's test results may be entered in the **SICKLECHECK™** Patient Test Data Sheet card provided with the kit and preserved for future records.

PICTORIAL PROCEDURE



INTERPRETATION OF RESULTS

- At the end of 15 minutes record the test result as noted in the table below.

	**Normal** Presence of colored band in control region (C) and test region (S).
	**Sickle Cell Disease (SCD) / Sickle cell with other hemoglobinopathies** The presence of coloured band in the control region (C) and test region (A).
	**Sickle Cell Trait or its association with other hemoglobinopathies** The presence of colored band in the control region (C) and no colored band at S & A.
	**Other Hemoglobinopathies or Thalassemia or other hemoglobinopathies associated with Thalassemia** The presence of colored band in the control region (C), test region (S) and test region (A). Such samples are to be confirmed by Hb electrophoresis or HPLC methods.
	**Invalid Results** The test is said to be invalid if no band appears at the control region (C) irrespective of the colored bands at the test region (S/A).

PERFORMANCE CHARACTERISTICS

SICKLECHECK™ was evaluated in comparison with similar rapid test for detection of sickle cell disorder as reference method using 100 samples. **SICKLECHECK™** demonstrated an accuracy of 100% in comparison with the reference method.

SICKLECHECK™ has been evaluated against reference test method of High performance liquid chromatography (HPLC) method by three reputed medical research institutes in India. Findings are as follows:

External evaluation I: Evaluation carried out with 463 samples.

Sensitivity : 98.14%	Specificity : 99.03%
Positive predictive Value : 98.1%	Negative Predictive Value : 99.02%

External evaluation II: Evaluation carried out with 359 samples.

	Sensitivity	Specificity
For Disease - HbSS	97.92%	100%
For Trait - HbAS	99.07%	98.81%

External evaluation III: Evaluation carried out with 200 samples.

	Sensitivity	Specificity
For Disease - HbSS	100%	100%
For Trait - HbAS	100%	100%
Negative for sickle cell disorder	NA	100%

REMARKS & LIMITATIONS

1. **SICKLECHECK™** does not quantitate the amount of Hb S or Hb A present in the specimen.
2. External study of **SICKLECHECK™** has shown, no interference in result with samples having high persistence of foetal hemoglobin (Hb F).
3. Test is dependant on both concentration of total Hb and percentage of Hb S & Hb A present in the sample.
4. Heterophilic substances might give erroneous results.
5. Lipemic or icteric sample may give erroneous results.
6. Presence of control line only means that the migration of the test is occurred. It does not serve as confirmation of addition of sample.
7. The test is screening test and has to be confirmed by electrophoresis and HPLC.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

1. A Global Perspective on Sickle Cell Disease. Pediatric Blood Cancer. Aygun B, Odame I. 2012;56:386–90.
2. The management of sickle cell disease. Adams RJ et al. Natl Institutes Heal. 2002; 1–206.
3. Diagnosis of Sickle Cell Disease and Its Related Hemoglobin Disorders. Yang Y-M, Brigham S, Liu PI. In: Mankad VN, Moore RB, editors.
4. Sickle Cell Disease: Pathophysiology, Diagnosis, and Management. Westport: Praeger; 1992. p. 201–20.
5. The spatial epidemiology of sickle cell anemia in India. Carinna Hockham et al. Scientific reports (2018) 8:17685
6. Pathophysiological insights in sickle cell disease. Odievre, M et al. IJMR 134, 532–537 (2011).
7. Sickle cell Trait: A Benign state? Taiwo R. Kotila. Acta Haematol 2016; 136:147-151.
8. Hematological and clinical features of sickle cell diseases: A Review. Donald R. Harkness. Hemoglobin, 4(3&4), 313-334(1980).
9. Sickle Cell Anemia Hemoglobin: The Molecular Biology of the First "Molicular Disease"- The Crucial Importance of Serendipity. Vernon M. Ingram. Genetics 167: 1-7 (May 2001).
10. Human Hemoglobin: HUEHNS. E. R. J. med. Genet. (l965). 2, 48.
11. Data on file: Zephyr Biomedicals (A division of Tulip Diagnostics (P) Ltd.)

SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	Do not reuse
Manufacturer	IVD — *In vitro* Diagnostic Medical Device	Contains sufficient for <n> tests	Do not use if package is damaged
Use by	REF — Catalogue Number	DEVICE — Device	BUF — Buffer
LOT — Batch Number / Lot Number	This side up	PIPETTE — Disposable Plastic Specimen Transfer device	

Manufactured by:
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