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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 "Molecular Disease"- The Crucial Importance of Serendipity. Vernon M. Ingram. Genetics 167: 1-7 (May 2001).
10. Human Hemoglobin: HUEHNS, E. R. J. med. Genet. (1965). 2, 48.
11. Data on file: Zephyr Biomedicals.

#### SYMBOL KEYS

 Temperature Limitation	 Consult Instructions for use	 Date of Manufacture	 Do not reuse
 Manufacturer	 <i>In vitro</i> Diagnostic Medical Device	 Contains sufficient for <n> tests	 Do not use if package is damaged
 Use by	 Catalogue Number	 Device	 Assay buffer
 Batch Number / Lot Number	 This side up	 Disposable Plastic Sample Applicator	



Manufactured by:

**Zephyr Biomedicals**

A Division of Tulip Diagnostics (P) Ltd.

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# 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.

#### 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, severe pneumonia; they can experience a sickle cell crisis.

**SICKLECHECK™** is a rapid, competitive 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 (T1 & T2) 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 (T1 & T2) 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 (T1 & T2), therefore forming colored bands. The presence of colored band at the test regions (T1 & T2) 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 sample applicator.
- C. **BUF** : Assay buffer vials.
- D. Package insert.

 REF	508010010
 Σ	10 Tests

#### OPTIONAL MATERIALS REQUIRED

10µl calibrated micropipettes, micropipette tips, sterile blood lancet, stopwatch.

#### STORAGE AND STABILITY

The sealed pouches in the test kit and the kit components may be stored between 4°C to 30°C till the duration of the shelf life as indicated on the pouch/carton. DO NOT FREEZE.

#### NOTES

1. Read the instructions carefully before performing the test.

- For in vitro diagnostic use only. NOT FOR MEDICINAL USE. For professional use only.
- Do not use the kit beyond expiry date and do not re-use the test device.
- Do not intermix reagents from different lots.
- 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.
- Handle all specimens as if potentially infectious. Follow standard biosafety guidelines for handling and disposal of potentially infectious material.
- 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:

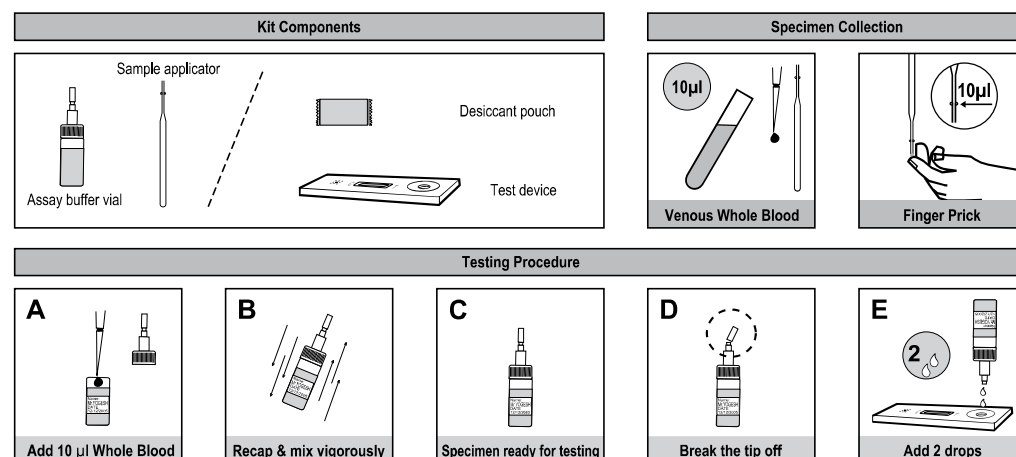
Collect whole blood in EDTA, Heparin, Sodium Citrate or ACD anticoagulant. Though fresh blood samples are preferable; the sample can be stored at 2°C to 8°C for up to 24 hours, in case of delay in testing. With the help of a micropipette / sample applicator transfer **10µl** whole blood in to the **labelled assay buffer vial** (provided with the kit) recap and mix vigorously. The specimen is now ready for 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 till blood oozes out freely. Wipe off the initial blood drop. Using a micropipette / sample applicator (provided with the kit) collect **10µl** whole blood for the test and immediately add to the **labelled assay buffer vial** (provided with the kit). Mix vigorously and the specimen is now ready for testing.

### TESTING PROCEDURE

- Bring the kit components of **SICKLECHECK™** devices to room temperature prior to testing.
- Open a foil pouch by tearing along the “notch” and remove the testing device.
- 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.**
- Label the device with specimen identity.
- Place the testing device on the flat horizontal surface.
- Mix the content of the labelled assay buffer vial.
- Hold the assay buffer vial in an upward position and break the tip off.
- Invert the vial and carefully dispense exactly **two drops** of specimen-buffer mixture into the specimen port (S).
- Observe the development of visible colored band at test regions (T1 & T2).
- Visible bands may be observed at the end of **15 minutes**, depending on the concentration of the analyte present in the specimen
- Do not read and interpret after 15 minutes.



### INTERPRETATION OF RESULTS

- At the end of 15 minutes record the test result as noted in the table below.
- Test line intensities may vary from faint to strong. Consider the faint lines also a visible band.

	<p><b>Invalid Result</b> 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 (T1/T2)</p>
	<p><b>Normal</b> Presence of strong intense colored band in control region (C) and test region (T1).</p>
	<p><b>Normal or Trait of other hemoglobinopathies</b> The presence of strong intense colored band in the control region (C), test region (T1) and/or weak intensity band at test region (T2)</p>
	<p><b>Sickle Cell Disease (SCD) / Sickle cell with other hemoglobinopathies</b> The presence of strong intense colored band in the control region (C), test region (T2) and/or weak intensity band at test region (T1)</p>
	<p><b>Sickle Cell Trait or its association with other hemoglobinopathies</b> The presence of strong intense colored band in the control region (C) and/or weak intensity band of test region (T2) and/or weak intensity band at test region (T1) and/or both (T1 &amp; T2)</p>
	<p><b>Other Hemoglobinopathies or Thalassemia or other hemoglobinopathies associated with Thalassemia</b> The presence of strong intense colored band in the control region (C), test region (T1) and test region (T2)</p>

### PERFORMANCE CHARACTERISTICS

**SICKLECHECK™** was evaluated in comparison with HPLC as reference method using 100 samples. **SICKLECHECK™** demonstrated an accuracy of 97% in comparison with the reference method.

### REMARKS & LIMITATIONS

- SICKLECHECK™** does not quantitates the amount of Hb S or Hb A present in the specimen.
- Test is dependant on both concentration of total Hb and percentage of Hb S & Hb A present in the sample.
- Heterophilic substances might give erroneous results.
- Lipemic or icteric sample may give erroneous results.
- Presence of control line only means that the migration of the test is occurred. It does not serve as confirmation of addition of sample.
- The test is screening test and has to be confirmed by electrophoresis and HPLC.
- Test does not work to its full potential if hemoglobin concentration is less than 5-8 gm%.
- If the hemoglobin concentration is between 5-8 gm% the sample volume should be doubled to 20 µl for testing.
- Due to the high percentage of Hb F the test is not recommended for testing newborns. It is recommended to test newborns after 3-4 months of age and the results should be confirmed with confirmatory test.
- SICKLECHECK™** has a detection limit of 20% for Hb S. Samples having Hb S below the detection limit might show erroneous results.

### 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

- 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.