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RESEARCH ARTICLE

# A Rapid Qualitative Point-of-Care Testing Device for Diagnosis of Sickle Cell Disorders

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## ABSTRACT

**Background:** Sickle cell disease (SCD) is a major hereditary hemoglobinopathy with high prevalence in central and southern India. Early detection remains a challenge in low-resource settings due to the dependence on expensive diagnostic systems such as high-performance liquid chromatography (HPLC) and electrophoresis. There is a global need for rapid, affordable point-of-care (POC) tests.

**Objective:** To develop, validate, and clinically evaluate a rapid, cost-effective lateral flow immunoassay (LFIA)–based diagnostic device (Biocard® Sickle Cell Rapid Test) for the detection of hemoglobin variants HbA and HbS

**Methods:** The study employed laboratory development of colloidal-gold-based LFIA, followed by analytical validation, reproducibility studies, inter- and intra-assay precision testing, accelerated and real-time stability evaluations, and analytical sensitivity/specificity assessment across three lots. A total of 290 pre-characterized clinical samples were evaluated at ICMR-NIIH and ICMR-CRMCH.

**Results:** The LFIA device showed >95% reproducibility and precision across three reagent lots. It reliably detected HbS at 1–2% concentration. Analytical specificity showed no cross-reactivity with HbF or other variants. Stability testing confirmed 27-month real-time stability at room-temperature. Clinical evaluation against HPLC demonstrated 100% sensitivity, 100% specificity, and 100% concordance across HbAA, HbAS, and HbSS patterns. Only 2.41% of HbSS samples showed faint adult Hb (A-band), likely due to transfusion or hemolysis.

**Conclusion:** The Biocard® Sickle Cell Rapid Test provides a robust, low-cost, and rapid diagnostic alternative suitable for POC settings, particularly in regions lacking laboratory infrastructure. Its excellent concordance with HPLC supports its integration into screening programs and resource-limited clinical settings.

**Keywords:** Sickle Cell Disease, Sickle Cell Trait, HbS, Lateral Flow Immunoassay, Point-of-Care Testing, Hemoglobinopathies, Rapid Diagnostic Test, India.

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## INTRODUCTION

Sickle cell disease (SCD) is a common recessively inherited multisystem blood disorder caused by sickle haemoglobin (HbS), which polymerizes and becomes insoluble under hypoxic conditions, causing a number of structural and functional abnormalities due to vaso-occlusion and hemolysis (Steele *et al*., 2019). Sickle cell anemia was first discovered in 1910 by James B. Herrick, who described the red cell as thin, sickle-shaped and crescent-shaped. It was later identified as a genetic disease because of a point mutation at position six of the β-globin gene located on chromosome 11. SCD is a group of hemoglobinopathies that produces two distinct mutant genotypes: homozygotes (SS) and heterozygotes (AS), resulting in distinct clinical syndromes such as sickle cell trait (HbAS), sickle cell disease (HbSS), sickle-Hb C Disease

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(HbSC), and sickle-Hb C Trait (HbAC) (Arishi *et al*., 2023; Randolph & Wheelhouse, 2012).

Sickle cell anemia (SCA) is the most common form of SCD and the etiology of Sickle cell disease is multifactorial, which contributes mostly to early childhood mortality (Arishi *et al*., 2023). In India, the prevalence of the sickle gene is found to be 0 to 18% in north eastern India, 0-33.5% in western India, 22.5 to 44.4% in central India, and 1 to 40% in southern India and the gene frequency of Hb-S varies between 0.031 to 0.41 (Gorakshakar, 2006). A recent initiative has been taken by the central government of India to eliminate sickle cell disease from central India (Madhya Pradesh), named as "Sickle Cell Anaemia Elimination Mission" on 1st July, 2023 (MoHFW, India, 2023a). Also, the Ministry of Health under the National Health Mission initiated the work on hemoglobinopathies (Thalassemia & Sickle Cell Disease) in 2016. The mission was to improve care of all sickle cell disease patients for their better future and to lower the prevalence of the disease through a multi-faceted coordinated approach towards screening and awareness strategies (MoHFW, India, 2023b).

The World Health Organization has recognized SCD as a global public health concern, with the incidence of infants born with sickle cell anemia estimated to be >400,000 and >5,000,000 with sickle trait per year worldwide (Hay *et al*., 2013; Piel *et al*., 2013). An accurate and early diagnosis is critically required for proper intervention, as SCA patients may die during childhood. There are several methods which have been considered as a gold standard in the diagnosis of SCD such as full count of blood cells, Hb electrophoresis, and high-performance liquid chromatography (HPLC) that can able to differentiate between normal, sickle cell trait, and SCA samples (Eastman *et al*., 1996; Adams *et al*., 2002; Fonseca *et al*., 2015; McGann & Hoppe, 2017). Other methods like mass spectrometry and genetic testing have also been widely used by health care professionals (Quinn *et al*., 2016; McGann & Hoppe, 2017).

These validated diagnostic kits are found to be sensitive and specific, but they need expensive equipment and a lot of training to use and thus are not great for places where resources are limited. Also, low-resource settings lack robust newborn screening programs for SCA. There are many treatments available at low cost, such as hydroxyurea and prophylaxis with antibiotics, immunization, exchange transfusions, oxygen, and pain management, but the clinicians do not have any low-cost diagnostic method to identify SCA patients in need of therapy (Randolph & Wheelhouse, 2012; Piety *et al*., 2015). The World Health Organization (WHO) and the National Institutes of Health have also called for technology development that can be deployed in low-resource settings (Smart *et al*., 2018). More research should focus on developing a rapid, inexpensive assay for SCD that could transform clinical care for affected persons in both low-income developing countries and urgent care settings. Therefore, the goal of this study was to develop and validate a diagnostic test having high accuracy, including the sensitivity, specificity, and limit of detection (LoD). We have developed a lateral flow immune assay (LFIA), which is easier to use and can be more affordable for places with fewer resources and the results are easily viewed at the point-of-care. Storage and transportation conditions are in the range of + 4 to ± 25ºC.

## METHODS

### Study design of developed kits

In this study, two different detection kits were used for rapid diagnosis of sickle cell disorders, i.e., the Biocard® Sickle Cell Rapid Test Kit and HI Speed Hemoglobin 'S' Solubility Test Kit. The Biocard® Sickle Cell Rapid Test Kit was designed as a rapid and highly sensitive test. It is a lateral flow chromatographic qualitative immunoassay to aid in the rapid diagnosis of sickle cell disorders of hemoglobin A, S, and C using fingerprick or venipuncture in whole blood samples. This is an *in-vitro* diagnostic test device and the results can be confirmed by naked eye in the POC setting for presumptive confirmatory of sickle cell hemoglobinopathies. Whereas, HI Speed Hemoglobin 'S' Solubility Test Kit is based on the principle of direct observation of turbidity, resulting from the insoluble sickle hemoglobin (HbS) in the presence of phosphate or sulfate buffer solution. This kit has a limitation over immunoassay by showing false positives in patients with erythrocytosis, hyperglobulinemia, extreme leukocytosis, or hyperlipidemia. Sometimes, some coarse flocculation may occur in these samples due to elevated levels of total serum protein.

### Biocard® Sickle Cell Rapid Test

*Test principle*

LFIA-based test has got two critical components, in this case, the first component is preparation of colloidal gold (nm) and conjugation with the capture Antibodies that would bind to the different variants of hemoglobins present in the blood samples.

*Process of preparation of colloidal gold*

Colloidal gold of particle size 40 nm for lateral flow assays was prepared by dissolving gold(III) chloride trihydrate ($HAuCl_4$) in deionized water to create a stock solution. The solution was heated to boiling, and trisodium citrate was added as a reducing agent, which initiated the formation of gold nanoparticles, marked by a color change from yellow to red or purple. The size of the nanoparticles was adjusted by varying the citrate-to-gold ratio to fit the required particle size of 40 nm. UV-vis spectroscopy was used to confirm the size and distribution of the nanoparticles, with an absorption peak observed at 520 to 530 nm. The colloidal gold was then stored in a sterile, light-protected container at 4°C to ensure stability. These nanoparticles were functionalized with specific antibodies or ligands for application in lateral flow assays.

*Conjugation of Variant Hemoglobin antibodies to Gold nanoparticles*

The conjugation process involved adjusting the pH of colloidal gold to 8 using 0.2 M $K_2CO_3$. A 0.1% blocker solution was prepared from a 10% stock and mixed gently with the gold

solution. The required concentration of variant haemoglobin antibody concentrations was added and mixed at a constant speed. A 1% BSA solution, diluted from a 10% stock, was then added to stabilize the nanoparticles, followed by another 30 minutes of mixing. The solution was centrifuged at 8000 rpm at 4°C for 50 minutes, and the supernatant was discarded. The pellet was resuspended in conjugate diluent. A 1:100 dilution of the conjugate was prepared and the OD was measured at 540 nm. The same protocol was followed for all the variant hemoglobin antibody conjugation to the gold particles.

*Process of preparation of coating on NCM*

The final volume of all the variant antibody-conjugated gold solutions was prepared by mixing the individually conjugated haemoglobin gold particles. This conjugated gold is stuck to the conjugate pad and assembled with the sample pad. Further, this is attached to NCM, having the Antibody coated on it. At the end of the assembly absorbent pad is attached to ensure the proper sample flow and absorb any extra liquid/solution.

Finally, these assembled uncut sheets (size) are cut into a single sheet of 3-4 mm (width) x 60 mm (length). This single uncut sheet is inserted into the cassettes, having slots for the insertion of the membrane. After the final assembly, these cassettes are sealed into the pouch in a humidity-controlled environment.

When the sample is added into the sample port, the different variants of haemoglobin present in the blood samples would bind to the respective antibodies present in colloidal gold on the conjugate pad. After binding this complex would flow towards the NCM, having the lines coated with different antibodies (A, S, C, Control). Due to the accumulation of colloidal gold at the site of the line coated, it will form a Red color.

*Test Procedure*

The Biocard® Sickle Cell Rapid Test requires a small amount of blood, ten microliters (10 µL), by fingerstick or venipuncture using the capillary sampler. The Sampler is placed into the buffer-loaded pre-filled extraction tube to release hemoglobin by lysing erythrocytes. About 2-3 drops of the treated sample are dropped from the pre-filled extraction tube and added to the sample inlet of the Sickle Cell card test. After that, the treated sample flows through the test cassette for 10 minutes before the result is read. The sample will interact with antibody-conjugated colorimetric detector nanoparticles and travel to the capture zones. A total of four detection lines are possible, with the control line appearing when the sample has been flowed through the card test. The presence of hemoglobin variants A, S, and C will be indicated by a red line in that region, as shown in Fig. 1.

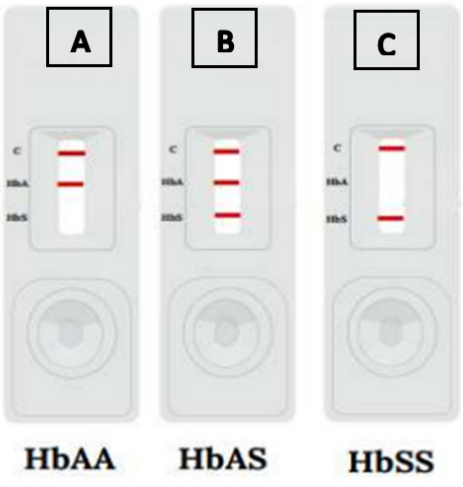


**Fig 1:** Sickle Cell card test represented four detection lines; (A) HbAA shows control line; (B) HbAS shows presence of hemoglobin variants A and S; (C) HbSS shows presence of hemoglobin variant S.

**In-House Method Validation**

All the assays mentioned below were assessed in 3 different lots and in triplicate to establish statistical significance. The method was validated on the following parameters**;**

*Reproducibility*

The reproducibility of the sickle cell disease assay was tested using 10 clinical samples and three different lots of reagents. Each sample was tested three times with each lot, resulting in a total of 90 tests. The same procedure was followed for all tests, and the testing order was randomized to avoid bias. Results were analyzed to evaluate consistency within each lot and between different lots using statistical methods. The test was considered successful if the variation within and between lots was small and the overall consistency exceeded 95%. This confirmed the assay's reliability for clinical use. The Lots used were RDSCR23004, RDSCR23005 and RDSCR23006.

*Inter Assay*

Inter-assay precision study involves the 3 different lots RDSCR23004, RDSCR23005 and RDSCR23006. The assay is performed in a day at 3 different time points for a single lot with 10 clinical samples. The results acquired are tabulated and the overall consistency between the sample results is analysed.

*Intra-assay*

The Intra-assay precision involves three different lots, RDSCR23004, RDSCR23005 and RDSCR23006. The assay is performed by three different personnel at a specified point in time and the results are recorded. About 10 clinical samples were used for each assay and the overall consistency of the results between personnel is recorded.

*Accelerated and real-time stability studies*

Accelerated and real-time stability testing were conducted to validate the performance and shelf life of the Sickle Cell lateral flow device (LFD). Accelerated testing exposed the device to elevated temperatures of RT, 30, 45, 60°C and 160 days with intervals of day 0, 4, 7, 16, 28, 36, 50, 65, 80, 95, 110, 125, 140 and 160 to simulate long-term storage conditions, assessing sensitivity, specificity, and physical integrity. Real-time testing was performed under recommended storage conditions, typically 2 to 8°C and RT (25°C), over the intended shelf life, with periodic evaluations at Day 0, 3 months,

6months, 9 months, 12 months, 15 months, 18 months, 21 months, 24 months and 27 months to confirm functional and chemical stability. Key metrics, including the ability to detect hemoglobin variants like HbS and HbA, were consistently monitored. Moisture- and oxygen-resistant packaging ensured environmental protection, and comprehensive stability data were documented in compliance with regulatory standards, confirming the device's reliability throughout its intended use.

*Analytical Sensitivity*

The analytical sensitivity of the sickle cell lateral flow device was assessed to determine its ability to detect specific concentrations of hemoglobin variants such as HbS and HbA. Validation involved testing samples with known proportions of these variants, establishing the device's detection limit, which reliably identified HbS at concentrations as low as 1 to 2% of total hemoglobin. Sensitivity was verified using standardized controls and clinical samples, including those from individuals with sickle cell disease (HbSS), sickle cell trait (HbAS), and normal hemoglobin profiles (HbAA).

*Analytical specificity*

The analytical specificity of the Sickle Cell lateral flow device was evaluated to confirm its ability to accurately identify target hemoglobin variants such as HbS and HbA without cross-reacting with non-target variants like HbF, HbC, or other blood components. Testing involved clinical samples with diverse hemoglobin profiles, including HbSC, HbSF, HbAS, and HbAA, to ensure the device could distinguish between normal and pathological profiles. Antibodies were designed to bind selectively to the target hemoglobins, minimizing cross-reactivity and false positives. A panel of samples other than Sickle cell disease was tested in 3 lots.

### *Ex-Vivo* Clinical Validation

The external performance evaluation of the Biocard Sickle Cell Rapid Test Card was conducted at the Indian Council of Medical Research–National Institute of Immunohaematology (ICMR-NIIH), Mumbai, Maharashtra, India, and the ICMR–Centre for Research Management and Control of Hemoglobinopathies (ICMR-CRMCH), Chandrapur, Maharashtra, India. A total of 290 clinical samples (n = 290) were evaluated as per the manufacturer's instructions. All samples were pre-characterized using high-performance liquid chromatography (HPLC, Bio-Rad Variant II) and included 78 normal samples (HbAA), 131 sickle cell disease samples (HbSS), and 81 sickle cell trait samples (HbAS). These characterized samples were subsequently used for validation of the developed rapid test kit.

## RESULTS AND DISCUSSION

### Result Interpretation of the Biocard Sickle Cell Rapid test

The results of the Biocard® Sickle Cell Rapid Test were interpreted based on the presence of specific detection lines. The control line served as a validity indicator, confirming proper sample flow and test functionality; its absence rendered the test invalid. Detection lines corresponding to hemoglobin variants A, S, and C provided diagnostic insights. A red line at A indicated normal hemoglobin (HbA), while a line at S signified the presence of sickle hemoglobin (HbS). A line at C indicated hemoglobin C (HbC). Various combinations of these lines differentiated conditions such as HbAA (normal), HbAS (sickle cell trait), HbSS (sickle cell disease), and HbSC (HbSC disease). Absence of any lines rendered the test invalid. All results were interpreted in conjunction with clinical evaluation, and inconclusive findings required confirmation through advanced diagnostic methods like HPLC.

### Internal Validation of Biocard Sickle Cell Rapid test

*Reproducibility*

The results of the sickle cell disease assay demonstrated high reproducibility across the three reagent lots (RDSCR23004, RDSCR23005, and RDSCR23006) when tested with 10 clinical samples (Table 1). Each sample was tested three times with each lot, resulting in a total of 90 tests. The variation within and between the lots was minimal, with consistency exceeding 95%. This confirmed the assay's reliability and accuracy for clinical use, as it consistently produced results in alignment with expected outcomes across different lots and sample types.

*Inter-Assay Precision*

The results of the inter-assay precision study demonstrated consistent performance across the three reagent lots - RDSCR23004, RDSCR23005, and RDSCR23006. The assay was performed at three different time points within a single day for each lot, using 10 clinical samples. The results were tabulated (Table 2), and the overall consistency between the sample results was analysed. The assay showed minimal variation between time points for each lot, indicating strong precision. This confirmed that the test was reliable and produced consistent results when performed multiple times within the same day with the same reagent lot.

*Intra-Assay Precision*

The results of the intra-assay precision study demonstrated minimal variation between the three different personnel who performed the assay using the three reagent lots - RDSCR23004, RDSCR23005, and RDSCR23006. Each of the 10 clinical samples was tested by all personnel, and the results were recorded. The overall consistency of the results across different operators was high, indicating that the assay showed strong intra-assay precision (Table 3). This confirmed that the test could be reliably performed by different personnel with consistent results.

*Accelerated and Real-Time Stability*

The results of the accelerated and real-time stability testing confirmed the Sickle Cell lateral flow device's reliability and stability throughout its intended shelf life. In the accelerated testing, the device was exposed to elevated temperatures of RT, 30, 45, and 60°C for up to 160 days, with evaluations at various intervals. Sensitivity, specificity, and physical integrity were consistently maintained across all temperatures, confirming that the device could withstand these conditions

**Table 1:** Reproducibility of the Kit results between 3 different lots

(Reproducibility test performed with 3 different lots )

| sample ID | Sample migration time in | Actual Results | | | RDSCR23004 | | | | RDSCR23005 | | | | RDSCR23006 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | T | | C | T | | Time | C | T | | Time | C | T | | Time |
| | | | 1 | 2 | | 1 | 2 | | | 1 | 2 | | | 1 | 2 | |
| samples | | | | | | | | | | | | | | | | |
| TVT/SCA/B/1 | < 3 mins | 4+ | 3+ | 0 | 4+ | 3+ | 0 | 65 sec | 4+ | 3+ | 0 | 66 sec | 4+ | 3+ | 0 | 63 sec |
| TVT/SCA/B/2 | | 4+ | 2+ | 0 | 4+ | 2+ | 0 | 70 sec | 4+ | 2+ | 0 | 71 sec | 4+ | 2+ | 0 | 72 sec |
| HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCS/B/1 | < 3 mins | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 71 sec | 4+ | 0 | 3+ | 74 sec | 4+ | 0 | 3+ | 73 sec |
| TVT/SCS/B/2 | | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 70 sec | 4+ | 0 | 3+ | 72 sec | 4+ | 0 | 3+ | 70 sec |
| HbA/HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCC/B/1 | < 3 mins | 4+ | 4+ | 3+ | 4+ | 4+ | 3+ | 73 sec | 4+ | 4+ | 3+ | 72 sec | 4+ | 4+ | 3+ | 73 sec |
| Negative Samples | | | | | | | | | | | | | | | | |
| TVT/N/B/120 | < 3 mins | 4+ | 0 | 0 | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 67 sec | 4+ | 0 | 0 | 66 sec |
| TVT/N/B/121 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 73 sec | 4+ | 0 | 0 | 72 sec |
| TVT/N/B/122 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 70 sec | 4+ | 0 | 0 | 72 sec | 4+ | 0 | 0 | 74 sec |
| TVT/N/B/123 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 56 sec | 4+ | 0 | 0 | 58 sec | 4+ | 0 | 0 | 57 sec |
| TVT/N/B/124 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 59 sec | 4+ | 0 | 0 | 60 sec | 4+ | 0 | 0 | 58 sec |

**Table 2A:** Inter assay precision performed with RDSCR23004 lot.

*(Inter day study performed with RDSCR23004)*

| Sample ID | Sample migration time in | Actual Results | | | DAY 1 | | | | DAY 2 | | | | DAY 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | T | | C | T | | Time | C | T | | Time | C | T | | Time |
| | | | 1 | 2 | | 1 | 2 | | | 1 | 2 | | | 1 | 2 | |
| **Samples** | | | | | | | | | | | | | | | | |
| TVT/SCA/B/1 | < 3 mins | 4+ | 3+ | 0 | 4+ | 3+ | 0 | 63 sec | 4+ | 3+ | 0 | 67 sec | 4+ | 3+ | 0 | 61 sec |
| TVT/SCA/B/2 | | 4+ | 2+ | 0 | 4+ | 2+ | 0 | 65 sec | 4+ | 2+ | 0 | 71 sec | 4+ | 2+ | 0 | 69 sec |
| HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCS/B/1 | < 3 mins | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 70 sec | 4+ | 0 | 3+ | 72 sec | 4+ | 0 | 3+ | 74 sec |
| TVT/SCS/B/2 | | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 73 sec | 4+ | 0 | 3+ | 68 sec | 4+ | 0 | 3+ | 71 sec |
| HbA/HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCC/B/1 | < 3 mins | 4+ | 4+ | 3+ | 4+ | 4+ | 3+ | 71 sec | 4+ | 4+ | 3+ | 72 sec | 4+ | 4+ | 3+ | 73 sec |
| Negative Samples | | | | | | | | | | | | | | | | |
| TVT/N/B/120 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 67 sec | 4+ | 0 | 0 | 62 sec |
| TVT/N/B/121 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec |
| TVT/N/B/122 | < 3 mins | 4+ | 0 | 0 | 4+ | 0 | 0 | 70 sec | 4+ | 0 | 0 | 72 sec | 4+ | 0 | 0 | 73 sec |
| TVT/N/B/123 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 58 sec | 4+ | 0 | 0 | 53 sec | 4+ | 0 | 0 | 58 sec |
| TVT/N/B/124 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 62 sec | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 59 sec |

without significant degradation. The real-time stability testing, conducted under recommended storage conditions (2–8°C and RT), showed no loss in functionality or chemical stability over the course of 27 months. Key metrics, including the detection of hemoglobin variants HbS and HbA, remained consistent throughout the testing periods. The moisture- and oxygen-resistant packaging effectively protected the device from environmental factors. These results demonstrated that the device retained its accuracy and reliability, meeting all regulatory standards for its intended shelf life.

**Table 2B:** Inter assay precision performed with RDSCR23005 lot.

*(Inter day study performed with RDSCR23005)*

| *Sample ID* | *Sample migration time in* | *Actual Results* | | | *DAY 1* | | | | *DAY 2* | | | | *DAY 3* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *C* | *T* | | *C* | *T* | | *Time* | *C* | *T* | | *Time* | *C* | *T* | | *Time* |
| | | | *1* | *2* | | *1* | *2* | | | *1* | *2* | | | *1* | *2* | |
| Samples | | | | | | | | | | | | | | | | |
| TVT/SCA/B/1 | < 3 mins | 4+ | 3+ | 0 | 4+ | 3+ | 0 | 63 sec | 4+ | 3+ | 0 | 67 sec | 4+ | 3+ | 0 | 61 sec |
| TVT/SCA/B/2 | | 4+ | 2+ | 0 | 4+ | 2+ | 0 | 65 sec | 4+ | 2+ | 0 | 71 sec | 4+ | 2+ | 0 | 69 sec |
| HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCS/B/1 | < 3 mins | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 70 sec | 4+ | 0 | 3+ | 72 sec | 4+ | 0 | 3+ | 74 sec |
| TVT/SCS/B/2 | | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 73 sec | 4+ | 0 | 3+ | 68 sec | 4+ | 0 | 3+ | 71 sec |
| HbA/HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCC/B/1 | < 3 mins | 4+ | 4+ | 3+ | 4+ | 4+ | 3+ | 71 sec | 4+ | 4+ | 3+ | 72 sec | 4+ | 4+ | 3+ | 73 sec |
| Negative Samples | | | | | | | | | | | | | | | | |
| TVT/N/B/120 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 67 sec | 4+ | 0 | 0 | 62 sec |
| TVT/N/B/121 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec |
| TVT/N/B/122 | < 3 mins | 4+ | 0 | 0 | 4+ | 0 | 0 | 70 sec | 4+ | 0 | 0 | 72 sec | 4+ | 0 | 0 | 73 sec |
| TVT/N/B/123 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 58 sec | 4+ | 0 | 0 | 53 sec | 4+ | 0 | 0 | 58 sec |
| TVT/N/B/124 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 62 sec | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 59 sec |

**Table 2C:** Inter assay precision performed with RDSCR23006 lot.

*(Inter day study performed with RDSCR23006)*

| *Sample ID* | *Sample migration time in* | *Actual Results* | | | *DAY 1* | | | | *DAY 2* | | | | *DAY 3* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *C* | *T* | | *C* | *T* | | *Time* | *C* | *T* | | *Time* | *C* | *T* | | *Time* |
| | | | *1* | *2* | | *1* | *2* | | | *1* | *2* | | | *1* | *2* | |
| Samples | | | | | | | | | | | | | | | | |
| TVT/SCA/B/1 | < 3 mins | 4+ | 3+ | 0 | 4+ | 3+ | 0 | 63 sec | 4+ | 3+ | 0 | 67 sec | 4+ | 3+ | 0 | 61 sec |
| TVT/SCA/B/2 | | 4+ | 2+ | 0 | 4+ | 2+ | 0 | 65 sec | 4+ | 2+ | 0 | 71 sec | 4+ | 2+ | 0 | 69 sec |
| HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCS/B/1 | < 3 mins | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 70 sec | 4+ | 0 | 3+ | 72 sec | 4+ | 0 | 3+ | 74 sec |
| TVT/SCS/B/2 | | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 73 sec | 4+ | 0 | 3+ | 68 sec | 4+ | 0 | 3+ | 71 sec |
| HbA/HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCC/B/1 | < 3 mins | 4+ | 4+ | 3+ | 4+ | 4+ | 3+ | 71 sec | 4+ | 4+ | 3+ | 72 sec | 4+ | 4+ | 3+ | 73 sec |
| Negative Samples | | | | | | | | | | | | | | | | |
| TVT/N/B/120 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 67 sec | 4+ | 0 | 0 | 62 sec |
| TVT/N/B/121 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec |
| TVT/N/B/122 | < 3 mins | 4+ | 0 | 0 | 4+ | 0 | 0 | 70 sec | 4+ | 0 | 0 | 72 sec | 4+ | 0 | 0 | 73 sec |
| TVT/N/B/123 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 58 sec | 4+ | 0 | 0 | 53 sec | 4+ | 0 | 0 | 58 sec |
| TVT/N/B/124 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 62 sec | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 59 sec |

*Analytical sensitivity*

The analytical sensitivity of the Sickle Cell lateral flow device was successfully validated, demonstrating its ability to detect hemoglobin variant HbS at concentrations as low as 1 to 2% of total hemoglobin. The validation process involved testing samples with known proportions of hemoglobin variants, including HbS and HbA, using both standardized controls and clinical specimens (Table 4). Clinical samples were sourced from individuals with sickle cell disease (HbSS), sickle cell trait (HbAS), and normal hemoglobin profiles (HbAA). The device reliably identified HbS across all tested scenarios, confirming its sensitivity and potential utility in diagnosing conditions associated with abnormal hemoglobin variants. No significant discrepancies or false results were observed during the assessment, ensuring the device's reliability for clinical application.

*Analytical Specificity*

The analytical specificity of the Sickle Cell lateral flow device was successfully validated, demonstrating its ability to accurately detect target hemoglobin variants, including HbS and HbA, without cross-reacting with non-target variants such as HbF, HbC, or other blood components. Clinical samples representing diverse hemoglobin profiles, including HbSC, HbSF, HbAS, and HbAA, were tested, and the device consistently distinguished between normal and pathological profiles (Table 5). The antibodies used in the device showed high selectivity, minimizing cross-reactivity and preventing false positives. Additionally, testing with a panel of non-sickle cell disease samples across three independent lots confirmed the device's reliability and robustness in maintaining specificity under varied conditions.

*Ex-Vivo Validation Results*

The external validation of the Biocard Sickle Cell Rapid Test Card was performed by the Indian Council of Medical Research (ICMR–NIIH, Mumbai and CRMCH, Chandrapur) using 290 clinical samples, including normal (AA) and sickle cell (AS and SS) patterns. The results obtained with the Biocard rapid test card were compared against the reference method, high-performance liquid chromatography (HPLC, Biorad Variant II). The Biocard rapid test demonstrated 100% sensitivity, specificity, and accuracy across all haemoglobinopathy patterns tested. A minor observation was noted in 2.41% (7/290) of sickle cell anemia samples, where a faint band was visible at the HA (adult Hb) position, potentially attributable to transfused or haemolyzed samples. Overall, the band intensities were clear and interpretable to the naked eye, and the rapid test results showed complete concordance with the gold standard HPLC method.

The evaluation confirmed the device's ability to accurately differentiate between various hemoglobin variants, validating its effectiveness under clinical conditions and its compliance with the manufacturer's specifications. No discrepancies were observed, reinforcing the robustness and diagnostic utility of the developed kits.

## DISCUSSION

Sickle cell disease (SCD) remains a significant global health challenge, particularly in low-resource settings where diagnostic capabilities and access to treatment are limited. This study successfully developed novel diagnostic methods: the Biocard® Sickle Cell Rapid Test Kit based on lateral flow immunoassay (LFIA), designed to provide rapid and cost-effective solutions for SCD diagnosis. The Biocard® Sickle Cell Rapid Test demonstrates promise for use at the point of care (POC) due to its ability to detect hemoglobin variants (HbA, HbS, and HbC) using a small blood sample. The lateral flow assay's reliance on colloidal gold conjugated to variant-specific antibodies enabled a robust and visually interpretable result within 10 minutes. Compared to established diagnostic methods like high-performance liquid chromatography (HPLC) and hemoglobin electrophoresis, this method is simpler, faster, and does not require sophisticated equipment or extensive technical expertise, making it highly suitable for deployment in resource-limited environments (Eastman et al., 1996; McGann & Hoppe, 2017). The HI Speed Hemoglobin 'S' Solubility Test is based on turbidity caused by the insolubility of HbS. While this method is less specific than LFIA, it provides a rapid preliminary assessment of sickle cell status. However, its limitations include potential false positives in patients with conditions such as erythrocytosis or hyperglobulinemia. This underscores the need for complementary diagnostic strategies in clinical practice (Piety et al., 2015; Randolph & Wheelhouse, 2012). The validation of these assays included rigorous testing across multiple clinical samples and reagent lots, demonstrating high reproducibility with >95% consistency across inter- and intra-assay studies. This ensures reliability and scalability for clinical implementation. Furthermore, stability studies confirmed the robustness of these assays under both real-time and accelerated conditions, with shelf-life evaluations supporting their viability in diverse environmental conditions, a crucial factor for their application in tropical and resource-constrained settings (Smart et al., 2018). This study aligns with global efforts by the World Health Organization (WHO) and National Institutes of Health (NIH) to address the unmet need for accessible SCD diagnostics in low-income countries. Existing diagnostic gold standards, although highly accurate, are limited by high costs and infrastructural requirements. The introduction of these low-cost, rapid tests represents a transformative shift, allowing for early detection, which is critical for initiating timely interventions and reducing childhood mortality associated with SCD (Piel et al., 2013). While the Biocard® Rapid Test and solubility test offer practical benefits, it is essential to recognize their diagnostic context. LFIA-based tests provide better sensitivity and specificity compared to solubility tests but may still fall short of the precision offered by HPLC or genetic testing. However, the trade-offs in cost, simplicity, and accessibility make these assays indispensable in settings where traditional methods are impractical. Studies have previously highlighted the need for POC diagnostics that balance accuracy with affordability,

**Table 3A:** Intra day assay performed with RDSCR23004 lot

*(Intra day study performed with RDSCR23004)*

| Sample ID | Sample migration time in | Actual Results | Forenoon | | | | | Midday | | | | Afterday | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | T | | C | T | | Time | C | T | | Time | C | T | | Time |
| | | | 1 | 2 | | 1 | 2 | | | 1 | 2 | | | 1 | 2 | |
| Samples | | | | | | | | | | | | | | | | |
| TVT/SCA/B/1 | < 3 mins | 4+ | 3+ | 0 | 4+ | 3+ | 0 | 63 sec | 4+ | 3+ | 0 | 67 sec | 4+ | 3+ | 0 | 61 sec |
| TVT/SCA/B/2 | | 4+ | 2+ | 0 | 4+ | 2+ | 0 | 65 sec | 4+ | 2+ | 0 | 71 sec | 4+ | 2+ | 0 | 69 sec |
| HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCS/B/1 | < 3 mins | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 70 sec | 4+ | 0 | 3+ | 72 sec | 4+ | 0 | 3+ | 74 sec |
| TVT/SCS/B/2 | | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 73 sec | 4+ | 0 | 3+ | 68 sec | 4+ | 0 | 3+ | 71 sec |
| HbA/HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCC/B/1 | < 3 mins | 4+ | 4+ | 3+ | 4+ | 4+ | 3+ | 71 sec | 4+ | 4+ | 3+ | 72 sec | 4+ | 4+ | 3+ | 73 sec |
| Negative Samples | | | | | | | | | | | | | | | | |
| TVT/N/B/120 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 67 sec | 4+ | 0 | 0 | 62 sec |
| TVT/N/B/121 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec |
| TVT/N/B/122 | < 3 mins | 4+ | 0 | 0 | 4+ | 0 | 0 | 70 sec | 4+ | 0 | 0 | 72 sec | 4+ | 0 | 0 | 73 sec |
| TVT/N/B/123 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 58 sec | 4+ | 0 | 0 | 53 sec | 4+ | 0 | 0 | 58 sec |
| TVT/N/B/124 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 62 sec | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 59 sec |

**Table 3B:** Intra day assay performed with RDSCR23005 lot.

*(Intra day performed with RDSCR23005)*

| Sample ID | Sample migration time in | Actual Results | Forenoon | | | | | Midday | | | | Afterday | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | T | | C | T | | Time | C | T | | Time | C | T | | Time |
| | | | 1 | 2 | | 1 | 2 | | | 1 | 2 | | | 1 | 2 | |
| Samples | | | | | | | | | | | | | | | | |
| TVT/SCA/B/1 | < 3 mins | 4+ | 3+ | 0 | 4+ | 3+ | 0 | 63 sec | 4+ | 3+ | 0 | 67 sec | 4+ | 3+ | 0 | 61 sec |
| TVT/SCA/B/2 | | 4+ | 2+ | 0 | 4+ | 2+ | 0 | 65 sec | 4+ | 2+ | 0 | 71 sec | 4+ | 2+ | 0 | 69 sec |
| HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCS/B/1 | < 3 mins | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 70 sec | 4+ | 0 | 3+ | 72 sec | 4+ | 0 | 3+ | 74 sec |
| TVT/SCS/B/2 | | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 73 sec | 4+ | 0 | 3+ | 68 sec | 4+ | 0 | 3+ | 71 sec |
| HbA/HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCC/B/1 | < 3 mins | 4+ | 4+ | 3+ | 4+ | 4+ | 3+ | 71 sec | 4+ | 4+ | 3+ | 72 sec | 4+ | 4+ | 3+ | 73 sec |
| Negative Samples | | | | | | | | | | | | | | | | |
| TVT/N/B/120 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 67 sec | 4+ | 0 | 0 | 62 sec |
| TVT/N/B/121 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec |
| TVT/N/B/122 | < 3 mins | 4+ | 0 | 0 | 4+ | 0 | 0 | 70 sec | 4+ | 0 | 0 | 72 sec | 4+ | 0 | 0 | 73 sec |
| TVT/N/B/123 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 58 sec | 4+ | 0 | 0 | 53 sec | 4+ | 0 | 0 | 58 sec |
| TVT/N/B/124 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 62 sec | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 59 sec |

**Table 3C:** Intra day assay performed with RDSCR23006 lot.

*(Intra day performed with RDSCR23006)*

| *Sample ID* | *Sample migration time in* | *Actual Results* | | | *Forenoon* | | | | *Midday* | | | | *Afterday* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *C* | *T* | | *C* | *T* | | *Time* | *C* | *T* | | *Time* | *C* | *T* | | *Time* |
| | | | *1* | *2* | | *1* | *2* | | | *1* | *2* | | | *1* | *2* | |
| Samples | | | | | | | | | | | | | | | | |
| TVT/SCA/B/1 | < 3 mins | 4+ | 3+ | 0 | 4+ | 3+ | 0 | 63 sec | 4+ | 3+ | 0 | 67 sec | 4+ | 3+ | 0 | 61 sec |
| TVT/SCA/B/2 | | 4+ | 2+ | 0 | 4+ | 2+ | 0 | 65 sec | 4+ | 2+ | 0 | 71 sec | 4+ | 2+ | 0 | 69 sec |
| HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCS/B/1 | < 3 mins | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 70 sec | 4+ | 0 | 3+ | 72 sec | 4+ | 0 | 3+ | 74 sec |
| TVT/SCS/B/2 | | 4+ | 0 | 3+ | 4+ | 0 | 3+ | 73 sec | 4+ | 0 | 3+ | 68 sec | 4+ | 0 | 3+ | 71 sec |
| HbA/HbS Positive Samples | | | | | | | | | | | | | | | | |
| TVT/SCC/B/1 | < 3 mins | 4+ | 4+ | 3+ | 4+ | 4+ | 3+ | 71 sec | 4+ | 4+ | 3+ | 72 sec | 4+ | 4+ | 3+ | 73 sec |
| Negative Samples | | | | | | | | | | | | | | | | |
| TVT/N/B/120 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 67 sec | 4+ | 0 | 0 | 62 sec |
| TVT/N/B/121 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec | 4+ | 0 | 0 | 71 sec |
| TVT/N/B/122 | < 3 mins | 4+ | 0 | 0 | 4+ | 0 | 0 | 70 sec | 4+ | 0 | 0 | 72 sec | 4+ | 0 | 0 | 73 sec |
| TVT/N/B/123 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 58 sec | 4+ | 0 | 0 | 53 sec | 4+ | 0 | 0 | 58 sec |
| TVT/N/B/124 | | 4+ | 0 | 0 | 4+ | 0 | 0 | 62 sec | 4+ | 0 | 0 | 68 sec | 4+ | 0 | 0 | 59 sec |

**Table 4:** Analytical sensitivity of the Sickle cell rapid device.

| *S. No.* | *Sample ID* | *Dilution Ratio* | *Obtained Results* | | |
|---|---|---|---|---|---|
| | | | *C* | *1* | *2* |
| 1 | Sickel Cell HbS positive sample TVT/SCS/B/1 | 1:1 | 4+ | 0 | 4+ |
| | | 1:2 | 4+ | 0 | 4+ |
| | | 1:4 | 4+ | 0 | 3+ |
| | | 1:8 | 4+ | 0 | 3+ |
| | | 1:16 | 4+ | 0 | 2+ |
| | | 01:32 | 4+ | 0 | 1+ |
| | | 1:64 | 4+ | 0 | 1+ |
| | | 1:128 | 4+ | 0 | Weak |
| | | 1:256 | 4+ | 0 | Very Weak |
| | | 1:512 | 4+ | 0 | 0 |
| 2 | Sickel Cell HbA/HbS positive sample TVT/SCC/B/1 | 1:1 | 4+ | 4+ | 4+ |
| | | 1:2 | 4+ | 4+ | 4+ |
| | | 1:4 | 4+ | 3+ | 3+ |
| | | 1:8 | 4+ | 3+ | 3+ |
| | | 1:16 | 4+ | 2+ | 2+ |
| | | 01:32 | 4+ | 1+ | 1+ |
| | | 1:64 | 4+ | 1+ | 1+ |
| | | 1:128 | 4+ | Weak | 1+ |
| | | 1:256 | 4+ | Very Weak | Weak |
| | | 1:512 | 4+ | 0 | 0 |

**Table 5:** Analytical Specificity of the Sickle cell device.

| *S. No* | *Sample ID* | *C* | *1* | *T* | *2* | *Inference* |
|---|---|---|---|---|---|---|
| 1 | TVT/HBA/PB/12 | 4+ | 0 | 0 | 0 | Negative |
| 2 | TVT/HBA/PB/18 | 4+ | 0 | 0 | 0 | Negative |
| 3 | TVT/HBA/PB/27 | 4+ | 0 | 0 | 0 | Negative |
| 4 | TVT/HBA/PB/33 | 4+ | 0 | 0 | 0 | Negative |
| 5 | TVT/HBA/PB/39 | 4+ | 0 | 0 | 0 | Negative |
| 6 | TVT/HBA/PB/41 | 4+ | 0 | 0 | 0 | Negative |
| 7 | TVT/HBA/PB/60 | 4+ | 0 | 0 | 0 | Negative |
| 8 | TVT/HBA/PB/84 | 4+ | 0 | 0 | 0 | Negative |
| 9 | TVT/HBA/PB/85 | 4+ | 0 | 0 | 0 | Negative |
| 10 | TVT/HBA/PB/91 | 4+ | 0 | 0 | 0 | Negative |
| 11 | TVT/LEK/PB/06 | 4+ | 0 | 0 | 0 | Negative |
| 12 | TVT/LEK/PB/10 | 4+ | 0 | 0 | 0 | Negative |
| 13 | TVT/LEK/PB/13 | 4+ | 0 | 0 | 0 | Negative |
| 14 | TVT/LEK/PB/29 | 4+ | 0 | 0 | 0 | Negative |
| 15 | TVT/LEK/PB/48 | 4+ | 0 | 0 | 0 | Negative |
| 16 | TVT/LEK/PB/64 | 4+ | 0 | 0 | 0 | Negative |
| 17 | TVT/LEK/PB/89 | 4+ | 0 | 0 | 0 | Negative |
| 18 | TVT/LEK/PB/110 | 4+ | 0 | 0 | 0 | Negative |
| 19 | TVT/LEK/PB/143 | 4+ | 0 | 0 | 0 | Negative |
| 20 | TVT/LEK/PB/167 | 4+ | 0 | 0 | 0 | Negative |
| 21 | TVT/ESR/PB/09 | 4+ | 0 | 0 | 0 | Negative |
| 22 | TVT/ESR/PB/45 | 4+ | 0 | 0 | 0 | Negative |
| 23 | TVT/ESR/PB/76 | 4+ | 0 | 0 | 0 | Negative |
| 24 | TVT/ESR/PB/90 | 4+ | 0 | 0 | 0 | Negative |
| 25 | TVT/ESR/PB/99 | 4+ | 0 | 0 | 0 | Negative |
| 26 | TVT/ESR/PB/124 | 4+ | 0 | 0 | 0 | Negative |
| 27 | TVT/ESR/PB/135 | 4+ | 0 | 0 | 0 | Negative |
| 28 | TVT/ESR/PB/153 | 4+ | 0 | 0 | 0 | Negative |
| 29 | TVT/ESR/PB/161 | 4+ | 0 | 0 | 0 | Negative |
| 30 | TVT/ESR/PB/178 | 4+ | 0 | 0 | 0 | Negative |

and the findings from this study align with these objectives (Fonseca et al., 2015; McGann & Hoppe, 2017). To maximize the utility of these diagnostic kits, integrating them into newborn screening programs and community-based healthcare initiatives should be prioritized. Further research should focus on refining the sensitivity of solubility tests, particularly to minimize false positives caused by interfering conditions. Additionally, expanding the scope of LFIA to detect other hemoglobinopathies could enhance its diagnostic coverage and clinical relevance (Hay et al., 2013).This study contributes significantly to the field of SCD diagnostics, offering practical, low-cost solutions that address the challenges of early diagnosis in low-resource settings. By bridging the gap between laboratory precision and field applicability, these diagnostic tools can support global health efforts to reduce the burden of SCD and improve patient outcomes. Continued collaboration between researchers, healthcare providers, and policymakers will be crucial to ensure widespread adoption and integration into healthcare systems.

## CONCLUSION

This study highlights the development of cost-effective and rapid diagnostic tools for sickle cell disease (SCD), particularly the Biocard® Sickle Cell Rapid Test Kit based on lateral flow immunoassay (LFIA), which offers a practical solution for low-resource settings. These tests, while not as precise as traditional methods like HPLC, provide a crucial alternative for early detection and timely intervention. Their simplicity, affordability, and reliability make them well-suited for implementation in resource-constrained environments, supporting global efforts to reduce SCD-related mortality. Continued refinement and integration into healthcare initiatives are essential to maximize their impact.

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