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SICKLECHECK™

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

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1.	African Journal of Laboratory Medicine,http://www.ajlmonline.org	1- 7
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Rapid test for simultaneous detection of Hb S and Hb A in human whole blood.

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Feasibility analysis of the SICKLECHECK™ test kit for rapid screening of sickle cell disease at a County Referral Hospital in Kenya

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Background: The burden of sickle cell disease in Western Kenya is substantial; however, there is limited research on the effectiveness of rapid diagnostic tests for the condition.

Objective: This study evaluated the feasibility of using the SICKLECHECK™ rapid test kit for detecting sickle cell disease at Bungoma County Referral Hospital, Kenya.

Methods: A cross-sectional study was carried out between October 2023 and February 2024 and included both healthy children and children with a known haemoglobin phenotype. The SICKLECHECK™ rapid screening test was compared to Bio-Rad™ high-performance liquid chromatography, which served as the reference standard. Sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were calculated using MedCalc™ statistical software.

Results: The study involved 194 children (98 girls and 96 boys), aged between 10 weeks and 15 years, with haemoglobin profiles sickle cell negative (n = 78), sickle cell trait (n = 21), and sickle cell disease (n = 95). The SICKLECHECK™ test demonstrated sensitivity, specificity, negative predictive value, and accuracy exceeding 97%, with a positive predictive value of 94.18% for haemoglobin A. It also effectively distinguished between normal (sensitivity 97.44%, specificity 99.14%), carrier (sensitivity 90.48%, specificity 98.27%), and disease (sensitivity 98.95%, specificity 98.99%) phenotypes.

Conclusion: Based on the findings in this study, SICKLECHECK™ could be a reliable point-of-care diagnostic tool for sickle cell disease. The encouragement of healthcare facilities, especially in resource-limited settings, to adopt the SICKLECHECK™ rapid test for routine screening and diagnosis of sickle cell disease is recommended.

What this study adds: This study highlights the diagnostic reliability of the SICKLECHECK™ rapid test in accurately identifying and differentiating sickle cell disease, trait, and normal haemoglobin phenotypes, reinforcing its potential role in strengthening early diagnosis efforts in clinical settings.

Keywords: sickle cell disease; rapid test; testing; accuracy; Kenya.

Introduction

Sickle cell disease (SCD) is a chronic, severely debilitating autosomal recessive disorder caused by a single-point mutation in the β-globin gene. Under hypoxic conditions, sickle haemoglobin undergoes polymerisation, leading to the reversible deformation of red blood cells into a sickle shape.[1] Globally, SCD is recognised as a major public health concern, with an estimated 300 000 to 400 000 infants born annually with the disease.[2] The burden is heaviest in low-resource settings, where access to early diagnosis and effective care remains limited.[3]

Sub-Saharan Africa accounts for approximately 75% of global SCD births,[4] and studies report that 50% to 80% of affected children in the region die before the age of five.[2] Screening of carriers in at-risk populations has been suggested since both parents as carriers carry a one in four likelihood of passing a homozygous offspring.[5] The World Health Organization has recently called on governments and international organisations to implement strategies for precise documentation of morbidity and mortality. This effort aims to address haemoglobinopathies, which are becoming more prevalent in society, posing a growing challenge to healthcare systems.[6] Although several advances have been made in molecular diagnostics, their applicability in resource-constrained settings remains limited.[7] There is a growing body of literature

emphasising the need for simplified, accessible, and scalable testing options of SCD, particularly in high-burden areas.[8] There is a need for an affordable and efficient method for accurate and early detection, given the high prevalence of haemoglobin anomalies in malaria-endemic regions such as Kenya.[5]

In Kenya, SCD and other haemoglobinopathies are highly prevalent, particularly in malaria-endemic regions such as Western Kenya and the coastal areas.[5] Recent data from Bungoma County show a sickle cell trait prevalence of 14.28% among blood donors.[9] Although homozygous SCD poses a significant burden in Western Kenya, studies on the effectiveness of rapid testing for the condition are scarce. This study assessed the practicality of using the SICKLECHECK™ test for rapid SCD screening at Bungoma County Referral Hospital, Kenya. This study specifically examined the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of SICKLECHECK™ in comparison to Bio-Rad™ high-performance liquid chromatography (HPLC), the reference method.

Methods

Ethical considerations

The study received approval from the Jaramogi Oginga Odinga Teaching and Referral Hospital Institutional Scientific and Ethical Review Committee (JOOTRH – ISERC) (reference number ISERC/JOOTRH/742/23). Authorisation to conduct the research was granted by the National Commission for Science, Technology, and Innovation (reference number NACOSTI/P/23/29577). Permission for data collection was obtained from Bungoma County Referral Hospital (reference number BDH/S&E/11/1/[256]). The study's objectives, procedures, potential risks, and benefits were clearly explained to both the participants and their parents or legal guardians. Written informed consent was obtained from the parents or legal guardians of all participants. Participation was entirely voluntary, and withdrawal from the study was permitted at any stage without penalty. To ensure data confidentiality, digital files were secured with password protection, while physical documents were stored in a locked and restricted-access location. No personally identifying information was collected or retained.

Study area

The study took place at Bungoma County Referral Hospital, Bungoma, Kenya, from 11 October 2023 to 22 February 2024. Bungoma County Referral Hospital is a level six hospital situated in the administrative centre of Bungoma County, Western Kenya. It serves as the primary referral facility for the county and its surrounding areas, providing healthcare to a population of 1 670 570 within a 2206.9 km² region.[10] Each year, around 3000 children attend the paediatric clinic at Bungoma County Referral Hospital, with SCD affecting approximately 5% of them, as reported in the hospital's records.[11] Sickle cell disease accounted for 7.8% of all paediatric admissions and was ranked among the top three causes of hospitalisation at Bungoma County Referral Hospital in 2023.[11]

Study design and population

This study followed an analytical cross-sectional design. Included were children who already had a known haemoglobin phenotype and were being monitored at the sickle cell clinic of Bungoma County Referral Hospital in Kenya, as well as children who were apparently healthy and visiting the maternal and child clinic of Bungoma County Referral Hospital in Kenya.

Sample size determination and sampling technique

The sample size for the study was determined using Fisher's exact test,[12] based on a previously reported prevalence of 14.8%.[9] Using this prevalence and standard parameters for statistical confidence and margin of error, the calculated sample size was approximately 194 participants, which was adopted for the study. A systematic random sampling method was employed, selecting every second child who met the inclusion criteria until the desired sample size was achieved.

Data collection

Recruitment and consent

The positive cases consisted of children aged 10 weeks to 15 years attending the sickle cell clinic at Bungoma County Referral Hospital with a known diagnosis of SCD (HbSS phenotype, i.e., homozygous for sickle cell haemoglobin) or sickle cell trait (HbAS phenotype, i.e., heterozygous for sickle cell haemoglobin), confirmed by prior haemoglobin electrophoresis or HPLC results. Healthy children within the same age range attending the Child Welfare Clinic for routine vaccinations were systematically selected as controls and presumed to be negative for SCD. Inclusion criteria for both groups included age between 10 weeks and 15 years, availability of haemoglobin profile for positive cases, and provision of informed consent by a parent or guardian. Exclusion criteria included recent blood transfusion (within the last 3 months), current use of antibiotics or hydroxyurea, presence of acute illness, or lack of consent. Participants were categorised into two groups, namely children with known SCD and healthy children without the disease. Parents or guardians received a detailed information sheet explaining the study objectives, participant selection process, procedures, potential risks and benefits, confidentiality measures, voluntary nature of participation, data sharing, and contact details for study-related inquiries. Following parental consent, children were assigned a unique identification number and included in the sample collection process.

Sample collection

For determination of the sickle cell phenotype, blood samples were collected from each participant. Approximately 2 mL of

whole blood was drawn into a vacuum collection tube containing ethylenediaminetetraacetic acid, gently inverted 8–9 times, and labelled with a unique participant identifier. The samples were promptly delivered to the laboratory. If not tested immediately, the samples were stored at 2 °C – 8 °C for up to 24 h. Prior to testing, stored samples were allowed to reach room temperature and were mixed thoroughly, according to the manufacturer's guidelines. Laboratory results were recorded using the study's standard data collection form.

Laboratory analysis

The performance of the SICKLECHECK™ (Zephyr Biomedicals, a division of Tulip Diagnostics (P) Ltd., Goa, India) rapid test kit for detecting sickle cell phenotypes was evaluated in comparison to the Bio-Rad™ (Bio-Rad Laboratories, Marnes-la-Coquette, France) HPLC method.

Sickle cell phenotype testing using SICKLECHECK™

SICKLECHECK™ operates on the principle of antibody/antisera agglutination with the corresponding antigen in a competitive immune-chromatography format. It utilises nanogold particles as the agglutination detection agent. The conjugate pad contains two components: a monoclonal antibody for haemoglobin S (HbS) and a monoclonal antibody for haemoglobin A (HbA), each conjugated to colloidal gold.

The monoclonal antibody conjugated with colloidal gold, specific to either HbS or HbA, binds to the corresponding antigens in the test specimen. As the specimen flows through the membrane assembly by capillary action, the antibody–antigen complex moves along the membrane. In the absence of HbS and HbA on the membrane, the complex continues to the test regions (T1 and T2), where no band forms because of a lack of binding. The absence of colour bands at the test regions (T1 and T2) indicates the presence of the corresponding antigens (HbS or HbA), respectively, in the specimen.

In the presence of HbS and HbA, the monoclonal antibody–colloidal gold conjugate moves along the membrane through capillary action, where it is captured by the respective antigen coated on the membrane (T1 & T2), resulting in the formation of colour bands. The appearance of colour bands at the test regions (T1 and T2) indicates that the specimen does not contain the corresponding antigens (HbS or HbA). The membrane's control region (C) is coated with goat anti-mouse immunoglobulin G (IgG), which causes any unbound colloidal gold conjugates to continue traveling along the membrane and become immobilised. The control band validates the test results and serves as a procedural control.

The components of the SICKLECHECK™ test kit were allowed to reach room temperature before use. The foil pouch was opened by tearing along the notch to remove the testing device. The desiccant pouch was checked to ensure it was blue; if colourless or pink, the test device was discarded and replaced. Once opened, the device was labelled with the participant's identity and used immediately.

The device was placed on a flat surface, and the assay buffer vial was mixed and held upright to break off the tip. The buffer, which lyses red blood cells to release haemoglobin and ensures proper sample flow and reaction, was mixed with the specimen. Two drops of this mixture were dispensed into the specimen port (S). Visible coloured bands at test regions (T1 and T2) were observed over a 15-min period, depending on analyte concentration. Tests were not read or interpreted after 15 min.

Test results were interpreted as follows: a result was deemed invalid if no band appeared in the control region (C), regardless of the presence of bands in the test regions (T1 or T2). A normal result was indicated by coloured bands in both the control (C) and test region T1. The presence of bands in the control (C) and test region T2 suggested SCD or sickle cell in combination with other haemoglobinopathies. A band in the control region (C) with no bands in either test region (T1 or T2) was interpreted as sickle cell trait or its association with other haemoglobinopathies. The appearance of bands in the control (C), test region T1, and test region T2 indicated the presence of other haemoglobinopathies, thalassaemia, or a combination of haemoglobinopathies with thalassaemia. To ensure internal quality control, each test batch included positive and negative control samples with known HbA, HbS, and mixed phenotypes, which were tested alongside participant samples. Additionally, the laboratory participated in an external proficiency testing programme coordinated by a collaborating external quality assurance provider.

Sickle cell phenotype testing using high liquid performance chromatography

The Bio-Rad™ HPLC was used to separate and quantify haemoglobin variants in whole blood based on the cation exchange principle. To prepare the sample, 5 µL of ethylenediaminetetraacetic acid anticoagulated blood was mixed with 1 mL of haemolysis solution provided in the kit. The instrument was then loaded with the diluted samples, which were further processed with the appropriate haemolysing/wash buffer before being inserted into the analytical cartridge. A programmed buffer gradient with increasing ionic strength was pumped through the cartridge, separating haemoglobin fractions based on their ionic interactions with the cartridge material. These fractions passed through a flow cell, where absorbance was measured at 415 nm, with a secondary wavelength of 690 nm to eliminate background noise. The software generated a printed chromatogram showing all eluted haemoglobin fractions and their respective retention times. Peaks were attributed to specific 'windows' based on retention times, with each peak corresponding to a haemoglobin component. If an unknown peak was observed, it was identified by its retention time. Each analysis cycle took approximately 6.5 min, from sample loading to the final printed results. If the HbS peak was present without any detectable HbA, the patient was diagnosed with SCD (i.e., HbSS phenotype). In contrast, the presence of both HbA and HbS peaks, with HbA being predominant, indicated sickle cell trait (i.e., HbAS phenotype). Commercially prepared Bio-Rad™

control samples, containing known haemoglobin variant compositions, were analysed daily alongside participant samples. The laboratory participated in an established external quality assurance programme coordinated by an accredited external body. As part of this programme, blinded proficiency testing samples were periodically analysed, and the results were submitted for assessment against established consensus values.

Data quality assurance

A trained phlebotomist was responsible for collecting the blood samples, ensuring the correct volume was obtained to maintain data quality. The samples were processed and tested according to the standard operating procedures of Bungoma County Referral Hospital's laboratory. Internal and external quality controls were consistently monitored and verified throughout the study. A second individual cross-checked the recorded results for accuracy. For enhanced validity and reliability, all statistical tests were performed using a Z-score of 1.96 and a 95% confidence level.

Data analysis

Data were stored using Microsoft Excel (Microsoft Corporation, Redmond, Washington, United States), while MedCalc™ statistical software (MedCalc Software Ltd, Ostend, Belgium) was used to calculate the sensitivity, specificity, PPV, NPV, and accuracy of SICKLECHECK™ in comparison to Bio-Rad™ HPLC, which served as the reference method. Results were presented in tables. The software computed sensitivity by dividing true positives by the total of true positives and false negatives, specificity by dividing true negatives by the sum of true negatives and false positives, PPV by dividing true positives by the sum of true positives and false positives, and NPV by dividing true negatives by the sum of true negatives and false negatives. Accuracy was calculated by multiplying sensitivity by the sum of prevalence and specificity, then multiplying by (1 – prevalence).

Results

Patient characteristics

A total of 194 children were included in the analyses, comprising 90 boys (46%) and 104 girls (54%) (Table 1). Participants ranged in age from 10 weeks to 15 years. Of these, 95 (49%) were confirmed to have SCD (HbSS phenotype), 21 (11%) were carriers of the sickle cell trait (HbAS phenotype), and 78 (40%) were sickle cell negative (HbAA phenotype, i.e., homozygous for haemoglobin A). Among the HbSS group, 47 (49%) were boys and 48 (51%) were girls. Of the children with the HbAS trait, nine (43%) were boys and 12 (57%) were girls. Among HbAA participants, 34 (44%) were boys and 44 (56%) were girls.

Detection of phenotypes

The SICKLECHECK™ assay correctly identified 98 out of 99 children with HbA as true positives (99.0%), and 94 out of 95 children without HbA as true negatives (98.9%). There was one false positive (1.1%) and one false negative (1.0%) for HbA detection (Table 2). For HbS detection, the test accurately identified 115 out of 116 children with HbS as true positives (99.1%), and 76 out of 78 without HbS as true negatives (97.4%). Two false positives (2.6%) and one false negative (0.9%) were observed for HbS detection (Table 2). The SICKLECHECK™ accurately identified 76 out of 78 children with HbAA as true positives (97.4%), and 115 out of 117 children without HbAA as true negatives (98.3%). There was one false positive (0.9%) and two false negatives (2.6%) for HbAA detection (Table 3). For HbAS detection, the test correctly identified 19 out of 21 children as true positives (90.5%), and 170 out of 173 children as true negatives (98.3%). Three false positives (1.7%) and two false negatives (9.5%) were recorded for HbAS detection (Table 3). In detecting HbSS, the test identified 94 out of 95 children as true positives (98.9%), and 98 out of 99 as true negatives (99.0%). There was one false positive (1.0%) and one false negative (1.1%) for HbSS detection (Table 3).

Efficacy of SICKLECHECK™

The SICKLECHECK™ assay showed a sensitivity of 97.98%, specificity of 98.95%, and overall accuracy of 98.80% for HbA detection (Table 4). The PPV for HbA was 94.18%, while the NPV was 99.65%. For HbS detection, sensitivity was 99.14%, specificity was 97.44%, and overall accuracy was 97.69%. The PPV for HbS was 89.04%, and the NPV was 99.85% (Table 4).

SICKLECHECK™ showed a sensitivity of 97.44% for detecting HbAA, 90.47% for HbAS, and 98.98% for HbSS (Table 5). Specificity was 99.14% for HbAA, 98.27% for HbAS, and 98.99% for HbSS. The PPVs were 95.15% for HbAA, 90.06% for HbAS, and 94.45% for HbSS. Negative predictive values were 99.55% for HbAA, 98.34% for HbAS, and 99.82% for HbSS. The overall accuracy was 98.89% for HbAA, 97.11% for HbAS, and 98.98% for HbSS (Table 5).

TABLE 1: Basic demographic characteristics of the study population, Kenya, 11 October 2023 – 22 February 2024.

Participant characteristics	All phenotypes			Haemoglobin phenotype								
				HbSS			HbAS			HbAA		
	Range	*n*	%	Range	*n*	%	Range	*n*	%	Range	*n*	%
All participants	-	194	100	-	95	49	-	21	11	-	78	40
Gender												
Male	-	90	46	-	47	49	-	9	43	-	34	44
Female	-	104	54	-	48	51	-	12	57	-	44	56
Age	10 weeks – 15 years	-	-	5 months – 15 years	-	-	6 months – 14 years	-	-	10 weeks – 14 years	-	-

HbAA, haemoglobin phenotype homozygous for haemoglobin A (sickle cell negative); HbAS, haemoglobin phenotype heterozygous for sickle cell haemoglobin (sickle cell trait); HbSS, haemoglobin phenotype homozygous for sickle cell haemoglobin (sickle cell disease).

TABLE 2: SICKLECHECK™ detection of haemoglobin A and haemoglobin S compared to Bio-Rad™ high-performance liquid chromatography, Kenya, 11 October 2023 – 22 February 2024.

Results	Bio-Rad™ HPLC		
	Positive	Negative	Total
SICKLECHECK™ HbA			
Postive	98†	1‡	99
Negative	1§	94¶	95
Total	99	95	194
SICKLECHECK™ HbS			
Positive	115†	2‡	117
Negative	1§	76¶	77
Total	116	78	194

HbA, haemoglobin A; HbS, haemoglobin S; HPLC, high-performance liquid chromatography.
†, TP, true positive; ‡, FP, false positive; §, FN, false negative; ¶, TN, true negative.

TABLE 3: SICKLECHECK™ detection of haemoglobin phenotypes AA, AS and SS compared to Bio-Rad™ high-performance liquid chromatography, Kenya, 11 October 2023 – 22 February 2024.

Result	Bio-Rad™ HPLC		
	Positive	Negative	Total
SICKLECHECK™ HbAA			
Postive	76†	1‡	77
Negative	2§	115¶	117
Total	78	116	194
SICKLECHECK™ HbAS			
Postive	19†	3‡	22
Negative	2§	170¶	172
Total	21	173	194
SICKLECHECK™ HbSS			
Postive	94†	1‡	95
Negative	1§	98¶	99
Total	95	99	194

HbAA, haemoglobin phenotype homozygous for haemoglobin A (sickle cell negative); HbAS, haemoglobin phenotype heterozygous for sickle cell haemoglobin (sickle cell trait); HbSS, haemoglobin phenotype homozygous for sickle cell haemoglobin (sickle cell disease). HPLC, high-performance liquid chromatography.
†, TP, true positive; ‡, FP, false positive; §, FN, false negative; ¶, TN, true negative.

TABLE 4: Sensitivity and specificity of the SICKLECHECK™ assay for the detection of haemoglobin A and haemoglobin S, Kenya, 11 October 2023 – 22 February 2024.

Diagnostic assessment	Haemoglobin A		Haemoglobin S	
	Value (%)	95% CI	Value (%)	95% CI
Sensitivity (TP/[TP+FN])	97.98	92.89–99.75	99.14	95.29–99.98
Specificity (TN/[TN+FN])	98.95	94.27–99.97	97.44	91.04–99.69
PPV (TP/[TP+FP])	94.18	69.70–99.13	89.04	63.10–96.35
NPV (TN/[TN+FN])	99.65	98.62–99.91	99.85	98.93–99.98
Accuracy (sensitivity × prevalence + specificity × 1-prevalence)	99.80	96.07–99.82	97.69	94.45–99.30

Note: These values were dependent on disease prevalence of 14.28% in Bungoma County. The sensitivity, specificity, PPV, NPV and accuracy for HbA and HbS phenotypes were evaluated by the open Epi Info™ CDC statistical software.
CI, confidence interval; FP, false-positives; FN, false-negatives; TP, true-positives; TN, true-negatives; PPV, positive-predictive value; NPV, negative-predictive value.

Discussion

The current study demonstrated that the SICKLECHECK™ assay achieved over 97% sensitivity, specificity, NPV, and accuracy in detecting HbA and HbS variants, as well as all relevant haemoglobin phenotypes (normal, carrier, and disease). The findings are consistent with a study conducted in Ghana and the United States and published in 2019,[13] which assessed the HemoTypeSC™ rapid testing method, reporting sensitivity and specificity above 99%, with an accuracy of 99.80% for detecting HbA and 97.69% for HbS. Similarly, the current results on the NPV for HbS agree with the same study,[13] which documented a NPV of 99.8% for HbS. The present findings are also consistent with those of a study conducted in India and published in 2024,[14] which evaluated a point-of-care rapid diagnostic test kit for screening SCD. The study reported the following performance metrics: for HbAA, a sensitivity of 98.04%, specificity of 99.35%, PPV of 99.50%, and NPV of 97.47%; for HbAS, a sensitivity of 99.07%, specificity of 98.81%, PPV of 97.25%, and NPV of 99.60%; and for HbSS, a sensitivity of 97.92%, specificity of 100%, PPV of 100%, and NPV of 99.60%. The current findings on accuracy are in agreement with a study conducted in the United States and published in 2016,[15] that validated the diagnostic performance of the Sickle SCAN™ test, reporting an overall accuracy of 99% at the bedside. The current specificity results also align with those reported by a study conducted in Nigeria and published in 2019,[16] which found that the HemoTypeSC™ test for sickle cell anaemia had a specificity of 99.9% under optimal field conditions. However, the current findings slightly differ in terms of sensitivity, which was somewhat lower at 93.4%. The current specificity findings also align with those of a study conducted in the United States and published in 2017,[17] which developed and evaluated a competitive lateral flow assay, reporting a specificity of 100% for diagnosing sickle cell anaemia. However, while the current study reported a higher specificity, the United States study had a slightly lower specificity of 90.0%, indicating a modest difference in diagnostic accuracy. These findings also correspond with those of a study conducted at Cincinnati Children's Hospital Medical Center, United States, and published in 2016,[18] which evaluated the effectiveness of a rapid, point-of-care lateral flow immunoassay for diagnosing SCD. The study reported a sensitivity of 99.5% for HbS, and 98.3% for HbA. However, the current results show slight differences in specificity, which were 92.5% for HbS, and 94.0% for HbA. The present findings also contradict those of a study conducted in Haiti and published in 2019,[19] which reported a sensitivity of 90.0% for Sickle SCAN™. The present findings also contradict those of a study conducted in Mali and published in 2024 on the comparative performance of two rapid diagnostic tests (Sickle SCAN™ and HemoTypeSC™).[20] The study reported sensitivities of 96.10% and 95.22%, respectively, for detecting the HbAS genotype, and 81.67% and 78.33%, respectively, for detecting the HbSS genotype.

The conflicting findings reported in some studies may be attributed to factors such as the variable interpretation of faint test bands,[13] suboptimal environmental conditions during field testing,[21] and reduced specificity in neonates because of high levels of foetal haemoglobin, which can interfere with antigen–antibody interactions in certain immunoassays.[15] These discrepancies highlight the importance of considering context-specific variables such as age, environmental testing conditions, and the regional prevalence of haemoglobin variants when assessing the performance of rapid diagnostic tests. Nevertheless, despite these occasional inconsistencies, the broader body of evidence, including the current study, strongly supports the effectiveness of lateral flow-based rapid diagnostics as reliable and scalable tools for screening SCD, particularly in low-resource settings.

TABLE 5: Sensitivity and specificity of the SICKLECHECK™ assay for the detection of haemoglobin HbAA, HbAS and HbSS, Kenya, 11 October 2023 – 22 February 2024.

Diagnostic assessment	Haemoglobin phenotype					
	HbAA		HbAS		HbSS	
	Value (%)	95% CI	Value (%)	95% CI	Value (%)	95% CI
Sensitivity (TP/[TP+FN])	97.44	91.04–99.69	90.48	69.62–98.83	98.95	94.27–99.97
Specificity (TN/[TN+FN])	99.14	95.29–99.98	98.27	95.02–99.64	98.99	94.50–99.97
PPV (TP/[TP+FP])	95.15	73.60–99.28	90.06	74.53–96.56	94.45	70.76–99.17
NPV (TN/[TN+FN])	99.55	98.27–99.89	98.34	94.08–99.55	99.82	98.72–99.97
Accuracy (sensitivity × prevalence + specificity × 1-prevalence)	98.89	96.20–99.85	97.11	93.67–98.98	98.98	96.35–99.88

Note: These values were dependent on disease prevalence of 14.28% in Bungoma County. The sensitivity, specificity, PPV, NPV and accuracy for HbAA, HbAS and HbSS phenotypes were evaluated by the open Epi Info™ CDC statistical software.

CI, confidence interval; FP, false-positives; FN, false-negatives; TP, true-positives; TN, true-negatives; PPV, positive-predictive value; NPV, negative-predictive value HbAA, haemoglobin phenotype homozygous for haemoglobin A (sickle cell negative); HbAS, haemoglobin phenotype heterozygous for sickle cell haemoglobin (sickle cell trait); HbSS, haemoglobin phenotype homozygous for sickle cell haemoglobin (sickle cell disease).

While a few false positive and false negative results were noted in our study, such occurrences are not unusual and may arise from a combination of technical and biological factors. For instance, faint or borderline test bands in lateral flow immunoassays can complicate interpretation, potentially leading to misclassification.[13,22] Additionally, biological variability in haemoglobin expression, such as elevated levels of foetal haemoglobin in neonates, the presence of rare haemoglobin variants, or coexisting haemoglobinopathies, may influence the assay's ability to accurately detect specific haemoglobin types.[23] These factors can affect antibody binding or visual detection, contributing to diagnostic discrepancies.[13] Further investigation through larger, population-based studies is warranted to better understand and address these limitations.

The SICKLECHECK™ test demonstrates high sensitivity, specificity, and accuracy for detecting SCD and trait, comparable to other rapid point-of-care assays for SCD identification. Based on these results, the SICKLECHECK™ test is recommended as an effective rapid screening tool for haemoglobin variants and phenotypes.

SICKLECHECK™ offers significant benefits for testing in situations requiring minimal blood volumes. For example, it is particularly effective for testing infants who cannot provide sufficient blood through venipuncture, as it requires only 10 µL of blood from a finger or heel stick. Maintaining follow-up with patients after leaving healthcare facilities can be difficult, especially in rural areas. In many parts of rural Africa, including Kenya, haemoglobin screening is typically conducted at centralised hospitals or laboratories, necessitating the transfer of diagnostic specimens away from the point of care. This approach results in increased costs, logistical complexities, and delays in obtaining test results.[13] Rapid point-of-care screening methods, such as SICKLECHECK™, effectively address these challenges. SICKLECHECK™ does not rely on instruments, electricity, or refrigeration, requires minimal training for operators, and provides results through visual inspection within 15 min. These features make it a cost-effective, fast, and accurate diagnostic tool in low-resource settings with high rates of SCD. With its substantial sensitivity and specificity, low sample volume requirements, independence from specialised equipment, ease of use, and rapid results, SICKLECHECK™ is a highly recommended tool for point-of-care screening of SCD and traits.

Recommendations

The study recommends that SICKLECHECK™ be adopted as a rapid point-of-care test for SCD and trait because of its minimal sample requirement, lack of need for specialised equipment or electricity, ease of use, and quick result turnaround.

Limitations

A limitation of our study was the inability to evaluate Haemoglobin C (HbC) detection using the SICKLECHECK™ test, as no participants with this variant were identified.

Conclusion

Based on the findings in this study, SICKLECHECK™ could be a reliable point-of-care diagnostic tool for SCD.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

A.S.K., P.M.M. and S.K.M. structured and participated in all aspects of the study. P.M.M. and S.K.M. oversaw the study. P.M.M. and A.S.K. performed data analysis and wrote the manuscript. S.K.M. evaluated the manuscript for logical contributions. All authors evaluated and approved the final manuscript for submission.

Sources of support

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Data availability

The data sets collected and analysed during the current study are not publicly available because of ethical reviewing guidelines, but they are available from the corresponding author, A.S.K., upon reasonable request.

Disclaimer

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RESEARCH ARTICLE

Evaluation of a point-of-care rapid diagnostic test kit (SICKLECHECK) for screening of sickle cell diseases

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Abstract

Sickle cell diseases (SCD) are the most common genetic disorders with significant morbidity and mortality worldwide, including in India. The high prevalence of this disorder in many geographical regions calls for the use of a point-of-care rapid diagnostic test (RDT) for early screening and management of the diagnosed cases to reduce the allied clinical severity. In view of this, the present study was undertaken for the validation of a point-of-care RDT kit (SICKLECHECK^{TM}) for the screening of SCD. This validation and diagnostic accuracy study was conducted among the cases advised for screening of SCD. For validation, all the recruited cases were investigated for both the SICKLECHECK^{TM} RDT kit and HPLC (Variant-II) considering HPLC as a gold standard. A total of 400 cases were screened for both tests. For the presence and absence of sickle cell hemoglobin in the samples, SICKLECHECK^{TM} RDT kit results showed a sensitivity and specificity of 99.39% and 98.73% respectively with references to HPLC findings. For the detection of the 'AS' pattern, the SICKLECHECK^{TM} RDT kit has shown a sensitivity and specificity of 99.07% and 98.81% respectively. For the detection of the 'SS' pattern, the SICKLECHECK^{TM} RDT kit has shown a sensitivity and specificity of 97.92% and 100.0% respectively. Cases with β thalassemia trait, hemoglobin E trait, hemoglobin Lepore trait and trait for hereditary-persistence-of-fetal-hemoglobin (high HbF %) diagnosed in HPLC were resulted with 'AA' pattern in SICKLECHECK^{TM} RDT kit. The high sensitivity and specificity of the SICKLECHECK^{TM} RDT kit insist on its use as a point-of-care screening tool for SCD especially where there is a lack of laboratory facilities as well as in hospital-based set-up requiring immediate diagnosis and management of SCD. However, for further confirmation, the samples should be analyzed with other gold standard techniques like HPLC.

Introduction

Sickle cell disease (SCD) is one of the most common monogenic inherited diseases in the world with an estimation of 111.91 per 1,00,000 live births across the globe [1]. With

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significant morbidity and mortality, SCD is also a major health burden in India contributing about 15% of SCD newborns of the world as well as ranked as the second worst affected country for SCD globally [2, 3]. In India, the prevalence of SCD is mainly specific to certain populations residing in various eastern, central and western regions as well as predominantly prevalent in socio-economically disadvantaged ethnic groups [4, 5]. Overall, the prevalence of the sickle cell trait in India varies from 1–40% in various communities [5].

Sickle cell disease is defined as the presence of sickle hemoglobin (HbS: *HBB*; c.20T>A, p. Glu6Val) that polymerizes under low oxygen conditions leading to the formation of a crescent or sickle shaped red blood cells [6]. The inheritance of Hb S occurs either in the heterozygous condition (sickle cell trait/sickle cell carrier/AS) or in homozygous condition (homozygous sickle cell anemia/SCA/SS) or in double heterozygous conditions with other hemoglobin variants. The cases with SCD are characterized by asymptomatic to severe clinical manifestations and the most clinical presentations are episodes of painful events, anemia requiring blood transfusion, acute chest syndrome, jaundice, stroke etc [7, 8].

At present many methods are there for the screening of SCD including the sickling slide test, solubility test, Hb electrophoresis, Isoelectric focusing, HPLC and genetic testing [9–11]. Most of these methods can only be used in laboratories requiring a supply of electricity, trained manpower as well as the maintenance of equipment which is very difficult in resource-poor setting especially when carried out in tribal-dominated areas. For the early diagnosis as well as management of SCD, now the world is focused on point-of-care test [12]. A number of point-of-care rapid diagnostics test (RDT) kits are available for the diagnosis of SCD including HemoTypeSC, SickleSCAN, SickleDex, solubility test etc. with significant sensitivity and specificity. The use of RDT kits for the screening of SCD will be helpful for the early identification of SCD patients and the subsequent provision of comprehensive management to reduce the morbidity and mortality rate related to SCD. Further, the use of RDT kits does not require any sophisticated laboratory set-up. In this regard, the present study was undertaken for the validation as well as the usefulness of another point-of-care RDT kit (SICKLECHECK^{TM}) for the screening of SCD.

Materials and methods

This was a validation, diagnostic accuracy study of the SICKLECHECK^{TM} RDT kit conducted among the patients advised for the screening of SCD. All the patients (more than 6 months of age) recruited during June 2023 to August 2023 for the screening of SCD were considered for this study. In an earlier hospital-based study carried out in this Medical College, the SCD cases was reported in around 50% of cases [13]. Considering the hypothesized sensitivity of 0.95, SCD prevalence of 0.50 and absolute precision of 0.05, the minimum number of SCD cases (sample size) required for this study was 146 [14]. However, a total sample size was fixed to 400 for the recruitment of normal individuals (Hb AA) for analysis.

After obtaining written informed consent from the patients (from the parents/guardian in case of a minor), 1–2 mL of venous blood samples were collected in an EDTA vial for laboratory investigations. All the collected blood samples were analyzed for both HPLC (Variant-II, Bio-Rad Laboratories, CA, USA) using β thalassemia short program and a rapid diagnostic test kit (SICKLECHECK^{TM}, Zephyr Biomedicals- A division of Tulip Diagnostics (P) Ltd., Goa, India) for the screening of SCD simultaneously. This study was approved by the Institutional Ethical Committee of M.K.C.G Medical College, Berhampur, Odisha (Ref No1298/Chairman-IEC, M.K.C.G.Medical College, Brahmapur-4, Date 05. 04.2023)

SICKLECHECK^{TM} RDT kit is a point-of-care, rapid, qualitative, immuno-chromatographic assay for the simultaneous detection of Hb S and Hb A in human blood samples.

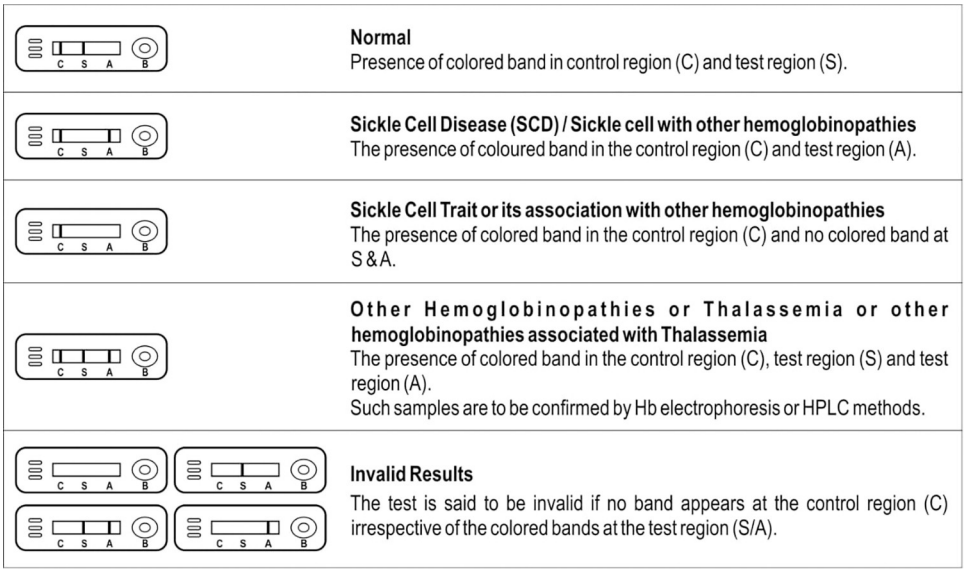


Fig 1. Interpretation of results of SICKLECHECK^{TM} rapid diagnostic kit for the diagnosis of sickle cell diseases.

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It is based on the principle of agglutination of antibodies/antisera with respective antigens in a competitive immuno-chromatography format along with the use of nanogold particles as agglutination revealing agents. The designed test kit pad is impregnated with two components that is, monoclonal antibody for HbS conjugated to colloidal gold and monoclonal antibody for Hb A conjugated to colloidal gold. As the test sample flows through the membrane assembly of the kit, the highly specific monoclonal antibody for Hb S and Hb A conjugated with colloidal gold make complexes with the respective Hb S and Hb A antigens present in the test sample and travels on the membrane due to capillary action. The complex travels on the membrane to the test region (S and A mark in the kit) where it is not captured by Hb S and Hb A coated on the membrane, thus forming no bands. The absence of colour bands at the test regions (S and A) indicates the presence of respective HbS and HbA antigens in the test samples and vice versa. The unbound colloidal gold conjugates move further on the membrane and are immobilized by the agglutinating sera for goat anti-mouse IgG coated on the membrane at the control (C mark in the kit). This control band acts as a procedural control and serves to validate the test results. The detailed interpretation of results generated from the-SICKLECHECK^{TM} RDT kit is shown in Fig 1.

This SICKLECHECK^{TM} RDT kit contains membrane test assembly, plastic specimen transfer devices with a 10 μL mark, assay buffer vials and sterile lancets. The test can be performed either in venous blood or by finger prick. In both case, 10 μL of blood samples was mixed with supplied assay buffers by gently shaking the vial sideways. Two drops of the specimen were applied into the specimen port (B) of the membrane device and after 10–15 minutes the result was interpreted by observing the clear bands in the C, S and A regions respectively.

Limit of detection

To analyze the minimum Hb S % that can be detected by SICKLECHECK^{TM} RDT kit, a serial dilution method was applied. For this, 5 different blood samples with 'AS' pattern and Hb S % of 20–30% in HPLC were considered and diluted with the normal ('AA' pattern) samples. These samples were diluted in 5:5, 4:6, 3:7, 2:8, 1:9 and 0.5:9.5 ration with the normal samples

and these diluted samples were simultaneously analyzed both in HPLC and SICKLECHECK™ RDT kit.

Data analysis

The characteristics of the study participants were described using descriptive statistics. The generated results of both test were analyzed by respective instruction manuals and categorized the cases accordingly. The diagnostic accuracy of the SICKLECHECK™ RDT kit was compared with HPLC results as the gold standard by analyzing sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using 2023 MedCalc Software Ltd. (http://www.medcalc.org/calc/diagnostic_test.php).

First, the diagnostic accuracy of the SICKLECHECK™ RDT kit was performed by analyzing the presence and absence of Hb S in the samples as follows

1. For HPLC
   - Presence of HbS: Cases with 'AS', 'SS', sickle cell and β thalassemia (HbS- β thalassemia), and SS with a history of blood transfusion.
   - Absence of HbS: Cases with 'AA', 'β thalassemia trait', 'β thalassemia major', 'hemoglobin E trait', 'hemoglobin Lepore trait', and 'trait for hereditary persistence of fetal hemoglobin'.
2. For SICKLECHECK™ RDT kit
   - Presence of Hb S: Cases with 'AS' and 'SS' pattern
   - Absence of Hb S: Cases with 'AA' and 'Others'(other hemoglobin variants).

Secondly, as theSICKLECHECK™ RDT kit is based on the screening for the SCD only, cases with 'AA', 'AS' and 'SS' patterns detected in HPLC were only considered for the diagnostic accuracy of the SICKLECHECK™ RDT kit.

Results

During the study period, a total of 400 unknown samples were included in this study. The median age of the study participants was 19 years with a range from 7 months to 70 years. There were 204 females and 196 males. For the diagnosis of SCD in all the recruited cases both HPLC (Variant-II) and SICKLECHECK™ RDT were carried out simultaneously. In SICKLECHECK™ RDT, a total of 228, 110, 56 and 6 cases were detected as 'AA', 'AS', 'SS' and 'Others' patterns respectively. On comparison of both the investigations, various hemoglobin disorders including normal (AA), β thalassemia trait, hemoglobin E trait, hemoglobin Lepore trait and trait for hereditary persistence of fetal hemoglobin (high HbF %) diagnosed in HPLC were resulted with 'AA' pattern in SICKLECHECK™ RDT kit. Similarly, the 'AS' pattern and trait for hereditary persistence of fetal hemoglobin (high HbF %) diagnosed in HPLC resulted in the 'AS' pattern in the SICKLECHECK™ RDT kit. Cases with HbS-β thalassemia, 'SS' with a history of blood transfusion and 'SS' in HPLC resulted in 'SS' pattern in the SICKLECHECK™ RDT kit. All the β thalassemia major cases in HPLC were detected as 'Others' pattern in the SICKLECHECK™ RDT kit. The detailed comparative analysis of both HPLC and SICKLECHECK™ RDT kit is depicted in Table 1.

As per HPLC finding, Hb S was present in 164 cases and absent in rest 236 cases. Out of 164 cases with Hb S in HPLC, 163 cases had Hb S and a lone case had no Hb S in the SICKLECHECK™ RDT kit. Similarly, out of 236 cases with no Hb S in HPLC, 233 cases had no Hb S and 3 cases had Hb S in the SICKLECHECK™ RDT kit. With references to HPLC findings,

Table 1. Comparison of results from HPLC (Variant II) and SICKLECHECK™ RDT kit (n = 400).

SICKLECHECK™ RDT kit	HPLC (Variant-II)
AA (n = 228)	AA (n = 200)
	AS (n = 1)
	β thalassemia trait (n = 20)
	Hemoglobin E trait (n = 1)
	HemoglobinLepore trait (n = 1)
	Trait for hereditary persistence of fetalhemoglobin (n = 5)
AS (n = 110)	AA (n = 2)
	AS (n = 106)
	Trait for hereditary persistence of fetalhemoglobin (n = 1)
	SS (n = 1)
SS (n = 56)	SS (n = 47)
	S-β thalassemias (n = 5)
	SS with history of blood transfusion (n = 4)
Others (n = 6)	AA (n = 2)
	β thalassemia major (n = 3)
	Trait for hereditary persistence of fetalhemoglobin (n = 1)

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SICKLECHECK™ RDT kit results showed a sensitivity of 99.39% and a specificity of 98.73% to detect the presence of Hb S in the samples (Fig 2).

As the SICKLECHECK™ RDT kit is designed for the diagnosis of SCD only, we have calculated the sensitivity and specificity from 359 cases including 204 cases of the 'AA' pattern, 107 cases of the 'AS' pattern and 48 cases of the 'SS' pattern resulted in HPLC. The detailed distribution of 359 cases is shown in Table 2. The sensitivity and specificity of the SICKLECHECK™ RDT kit were analyzed separately for the 'AA', 'AS' and 'SS' pattern. For the detection of the 'AS' pattern, the SICKLECHECK™ RDT kit has shown a sensitivity of 99.07% and a specificity of 98.81%. For the detection of the 'SS' pattern, the SICKLECHECK™ RDT kit has shown a sensitivity of 97.92% and a specificity of 100.0%. For the detection of the 'AA' pattern, the SICKLECHECK™ RDT kit has shown a sensitivity of 98.04% and a specificity of 99.35%. The sensitivity, specificity, PPV and NPV of theSICKLECHECK™ RDT kit for the detection of sickle cell disease are depicted in Table 3.

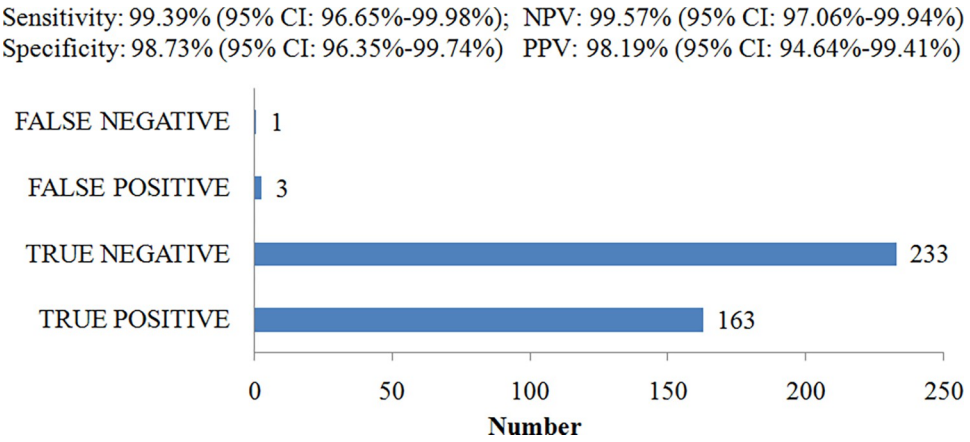


Fig 2. Sensitivity and specificity of SICKLECHECK™ RDT kit for the presence of sickle hemoglobin.

https://doi.org/10.1371/journal.pone.0309045.g002

Table 2. Distribution of cases with respect to HPLC (Variant-II) and SICKLECHECK™ RDT kit results (n = 359).

Sickle Cell Diseases		SICKLECHECK™ RDT kit				Total
		'AA' pattern	'AS' pattern	'SS' pattern	'Others' pattern	
HPLC Results	AA	200 (98.04%)	2 (0.98%)	0	2 (0.98%)	204
	AS	1 (0.94%)	106 (99.36%)	0	0	107
	SS	0	1 (2.08%)	47 (97.92%)	0	48
Total		201	109	47	2	359

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Table 3. SICKLECHECK™ RDT kit sensitivity, specificity, positive predictive value and negative predictive value in comparison to HPLC findings for the detection of sickle cell diseases.

	SICKLECHECK™ RDT kit					
	'AA' pattern		'AS' pattern		'SS' pattern	
	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	98.04	95.06–99.46	99.07	94.90–99.98	97.92	88.93–99.95
Specificity	99.35	96.46–99.98	98.81	96.56–99.75	100.00	98.82–100.00
Positive predictive value	99.50	97.26–99.99	97.25	91.98–99.09	100.00	92.45–100.0
Negative predictive value	97.47	93.65–99.31	99.60	97.25–99.94	99.68	97.81–99.95

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Table 4. Cases with conflicting results (n = 6).

Case No.	Results of SICKLECHECK™ RDT kit	Results of HPLC	Hemoglobin variants from HPLC (%)				Total hemoglobin level (mg/dL)
			HbA0	HbA2	HbF	HbS	
1	AS	AA	84.5	2.6	0.6	0	11.3
2	AS	AA	80.1	2.7	0.4	0	7.7
3	AS	SS	6.5	2.7	14.3	73.3	7.3
4	Other	AA	88.1	3.2	0.9	0	11.9
5	AA	AS	67.4	3.8	4.1	17.0	11.7
6	Other	AA	84.2	2.7	1.8	0	10.9

https://doi.org/10.1371/journal.pone.0309045.t004

In the comparison of both the HPLC and SICKLECHECK™ RDT kit in 359 cases, 6 cases showed conflicting results with respect to each test. As the SICKLECHECK™ RDT kit has certain limitations for lower hemoglobin level, high fetal hemoglobin level, percentage of both Hb S and Hb A in the samples etc., the detailed hemoglobin level along with HPLC finding of 6 contradictory cases is shown in Table 4. None of these cases had significant findings that can be correlated with the limitations of the SICKLECHECK™ RDT kit.

The limit of detection for SICKLECHECK™ RDT kit for the detection of Hb S in the blood samples were analyzed with a serial dilution and observed that samples with less than 3% of Hb S had an appearance of 'S' line indicating the absence of Hb S in the samples. So a limit of detection of 3% of Hb S in the samples may be considered for the detection of Hb S by SICKLECHECK™ RDT kit.

Discussion

During this validation study, the result of SICKLECHECK™ RDT kit was compared with the HPLC as a gold standard technique for the screening of SCD. In comparison, the

SICKLECHECK™ RDT kit was found to have high sensitivity and specificity for the screening of SCD. It showed high sensitivity and specificity of more than 98% in both when compared to either the presence or absence of Hb S in the samples; or the genotypes (AA, AS, SS) of the individuals respectively. These observations insist on the use of the SICKLECHECK™ RDT kit as a screening tool for SCD.

SICKLECHECK™ RDT kit can be used in remote areas without requiring sophisticated equipment, electricity, laboratory etc. Only minimum training is required for interpretation of results. Secondly, the results can be read within 15 minutes of the blood collection compared to 24–48 hours for HPLC (from sample collection to reporting) affirming its use in many hospital set-ups necessitating immediate diagnosis of the SCD for proper management and treatment. Further, the estimated price per kit is 1.3 USD, which is comparatively low than HPLC (~10 USD) and is also comparable to other point-of-care tests. In the state of Odisha with high prevalence of SCD as well as inhabitants of many tribal communities especially residing in remote areas with minimal or no health facilities, the SICKLECHECK™ RDT kit will be useful as a point-of-caretest for SCD. The use of point-of-care RDT for the screening of SCD will lead to early identification of disease and subsequent provision of comprehensive care. The comprehensive care management of SCD patients especially during childhood has been associated with a significant reduction in morbidity and mortality [15].

During the study, a number of other Hb variants were also reported and some of them were also co-inherited with Hb S. Interestingly, all the double heterozygous states of Hb S and β thalassemia diagnosed by HPLC were reported as 'SS' patterns in SICKLECHECK™ RDT kit. Again, all four 'SS' cases with a history of blood transfusion (HbA0 level was 10–16% in HPLC) were also reported as 'SS' pattern in the SICKLECHECK™ RDT kit. Both of these observations also affirm its use in other variants of SCD including double heterozygous state of Hb S and β thalassemia which is the second most prevalent severe phenotype of SCD after 'SS' genotype worldwide including India [16]. However, its pattern in another double heterozygous state with Hb S needs to be investigated further. Though, all the 'SS' cases with blood transfusion were resulted with 'SS' pattern, we recommend to test after 3 months of blood transfusion for any false negative results.

This SICKLECHECK™ RDT kit has also been designed for the diagnosis of other Hb variants. Except few, the majority of Hb variants including β thalassemia trait, hemoglobin E trait, hemoglobin Lepore trait and trait for hereditary persistence of fetal hemoglobin (high HbF %) diagnosed by HPLC in this study were resulted with 'AA' pattern in SICKLECHECK™ RDT kit. All three β thalassemia major cases in HPLC were detected as 'Others' patterns in the SICKLECHECK™ RDT kit. Thus, the diagnosis of other Hb variants (other than Hb S) by SICKLECHECK™ RDT kit needs to be revised requiring further upgradation of membrane biology. In such a finding, the samples should be analyzed by HPLC for further confirmation.

On estimating the limit of detection ofSICKLECHECK™ RDT kit, the serial dilution showed that the SICKLECHECK™ RDT kit can detect a minimum of 3% of Hb S in the samples. With a 3% of limit of detection in the present study, the SICKLECHECK™ RDT kit may be useful for the screening of SCD in newborn. However, further necessary evaluation is required for its use in newborns screening on SCD.

Two mostly validated and recommended RDT kit for the screening of SCD were SickleSCAN and HemoTypeSC. SickleSCAN assay employs the sandwich format chromatographic immunoassay using polyclonal antibodies specific to Hb A, Hb S and Hb C; while HemoTypeSC assay are based on the competitive lateral flow immuno-chromatographic assay using specific antibodies against Hb A, Hb S and Hb C [17, 18]. Both the RDT kits have been validated and evaluated in many studies for its wider use. Here, SICKLECHECK™ RDT kit used the same principle like HemoTypeSC, but there is a feasibility of detection of 'Other' variants

instead of Hb C alone. As Hb C variant is a rare Hb disorder in India with very few case reports, the detection of 'Other' variants is important as designed in SICKLECHECK^{TM}RDT kit in country like India where other Hb variants are highly prevalent including β thalassemia, Hb D^{Punjab}, Hb E etc.

The use of competitive immuno-chromatographic assay in the SICKLECHECK^{TM} RDT kit enhances the specificity of the test results by avoiding the possible interferences from other proteins in the samples as well as can allow the detection of a wide range of concentrations of target molecules (Hb A and Hb S). It may also exhibit lower background signals in the absence of the target Hb, which can contribute to improve the signal-to-noise ratios resulting better sensitivity in certain situations. Further this competitive immunoassaymay be a better option from the sandwich type immunoassay which are usually susceptible to the prozone effect (hook effect), in which a high concentrations of a particular Hb may leading to a saturation of the binding sites on the labelled conjugate and capture reagent on the test line, as a result, the excess Hb competes less effectively with the labelled conjugate, causing a decrease in the signal at the test line yielding a false negative results [19–21].

There are certain limitations of the study; (1) it does not quantify the amount of Hb S and Hb A in the samples; (2) The presence of heterophilic substances in the samples and lipemic or icteric samples may give incorrect results though no such type of samples was analyzed in this study. (3) The presence of a control line only means that the migration of the specimen is occurred. It does not serve as confirmation of the addition of more test specimens.

In conclusion, the SICKLECHECK^{TM} RDT kit can be used as a point-of-care test for the screening of SCD only. The readability of results in the SICKLECHECK^{TM} RDT kit is easy and clear. A clear and dark band in the test membrane should be considered for diagnosis; while the appearance of any faint or light bands should go for further confirmation with other gold standard techniques like HPLC.

Supporting information

S1 Dataset.
(XLSX)

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