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Assessment of Sickle SCAN® Test Performance for Abnormal Haemoglobins Characterisation in Abidjan, Côte d'Ivoire

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ABSTRACT

Background: Sickle cell disease is characterised by the presence of haemoglobin (Hb) S, which results from a structural abnormality of haemoglobin. This condition is a public health problem in large parts of the world, such as sub-Saharan Africa. The recent development of rapid detection tests (RDTs), which do not require advanced equipment or electricity, could make screening for this haemoglobinopathy more accessible in these sub-Saharan regions. The aim of our study was to evaluate the performance of the Sickle SCAN® test for the rapid detection of haemoglobins A, S and C. **Methodology**: After obtaining informed consent, blood samples were collected from three hundred patients. These patients came to the clinical biochemistry and haemobiology unit of Pasteur Institute of Côte d'Ivoire, Cocody site, for a biological check-up. We used these samples to screen for sickle cell disease using the Sickle SCAN rapid test® and compared the results with those of haemoglobin electrophoresis performed using a Sebia™ Hydrasis machine. We assessed the performance characteristics of Sickle SCAN® by evaluating 300 patients, including children and adults, seen at the clinical biochemistry and haemobiology unit of the Pasteur Institute of Côte d'Ivoire for biological check-ups.**Result:** Analysis of performance characteristics including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy showed values of 100% for each parameter. The analysis showed that the Sickle SCAN® test was highly consistent with the Sebia™ reference method, with robust results for the detection of the different genotypes. The Kappa coefficient and Receiver Operating Characteristic (ROC) curve confirmed the validitý of the Sickle SCAN® test as a reliable tool for rapid screening for sickle cell disease. No significant differences were observed between the results of the Sickle SCAN® test (test method) and those of the Sebia™ method (reference method). For the sickle cell disease diagnosis, the Sickle S SCAN test® could therefore represent a credible alternative. **Conclusion:** The study shows that the Sickle SCAN® rapid test can be validated as a tool for screening and/or rapid diagnosis of sickle cell disease in our environment. This does not rule out the need for a second confirmatory test.

Keywords: Sickle cell disease, screening, Sickle SCAN®, Abidjan, Côte d'Ivoire

I- INTRODUCTION

Sickle cell anaemia is a hereditary disease that mainly affects black people. Approximately 75% of children born with homozygous haemoglobin (SS) live in sub-Saharan Africa, and more than 50% of children with this disease die before the age of 5 **[1; 2].** In Côte d'Ivoire, studies have shown a prevalence of haemoglobin S between 12 and 14% in the general population according to Kakou-Danho et al. (2020), and around 12% in the infant population of Abidjan with a coexistence of AC (6.2%) and AFA2 or β-thalassaemia (2.7%) traits **[3; 4].**

Biological diagnosis of haemoglobinopathies is based on two principles. Firstly, the identification and quantification of variants whose charge or affinity for a support enables them to be separated into fractions and, secondly, assays of minor fractions **[5; 6]**.

While in developed countries, the diagnosis of haemoglobinopathies requires three separate phenotypic tests, including at least one electrophoresis technique **[7]**, in countries with limited resources, diagnosis uses only one technique, which is often not financially or geographically accessible. In addition, some laboratories continue to use the Emmel test, which is tedious, operator-dependent and obsolete. Furthermore, recent studies carried out in Côte d'Ivoire **[8]**, Nigeria **[9]**, Democratic Republic of Congo (DRC) **[10]** and United States **[11]** have demonstrated the feasibility and acceptability of rapid tests for the rapid screening of sickle cell disease, in particular for the identification of abnormal haemoglobins S and C.

The widespread use of these tests in developing countries would enable rapid screening for these haemoglobin profiles in rural health centers, which are generally a long way from major centers with dedicated equipment. In addition, these rapid tests are simple to perform and easy to transport in conditions that do not require any special arrangements.

The aim of this study is to evaluate the performance of the Sickle SCAN® test for the rapid screening of sickle cell disease in order to help to reduce the morbidity and mortality associated with this disease in both urban and rural areas.

II- METHODOLOGY

II- 1- Site and type of study

This is a prospective cross-sectional study which took place in November 2024 at the Pasteur Institute of Côte d'Ivoire, Cocody site.

II- 2- Study population :

The study involved three hundred (300) volunteers of both sexes who came to the clinical biochemistry and haemobiology unit of the Pasteur Institute of Côte d'Ivoire, Cocody site for a biological check-up. Participants ranged in age from 01 to 60 years. Apart from ethical considerations, the only non-inclusion criterion was a blood transfusion less than 120 days old.

II-3- Ethical considerations

This study was approved by the National Ethics Committee for Life Sciences and Health. Therefore, after being informed of the study, each participant was asked to give verbal and written consent prior to any sampling procedure.

II-4- Blood sampling

Venous blood (5 mL) was collected from each selected patient in a tube containing ethylene diamine tetra acetate (EDTA). This sample was screened using the Sickle S SCAN® rapid test and the Sebia™ Hydrasis automated test system. Samples for which the tests were not performed on the same day were kept refrigerated at 4°C for a maximum of one week.

II-5- Screening for abnormal haemoglobins

For each participant, a haemogram was first performed using an automated multi-parameter haematology analyser, the Abbott Cell-Dyn Rubby system. This was followed by haemoglobin electrophoresis using a Sebia™ Hydrasis machine, which was used as the reference test for comparison **[12].** Finally, the Sickle SCAN rapid screening test® (BioMedomics Inc., Durham, North Carolina, USA) was performed according to the manufacturer's instructions.

**** Principle of the haemoglobin electrophoresis test:***

This is the classic agarose gel test carried out using Sebia™' Hydrasis machine. Its principle is based on the fact that haemoglobin is a complex molecule made up of two pairs of polypeptide chains. Each chain is linked to the heme, a tetrapyrrolic nucleus (porphyrin) which chelates an iron atom. The heme part is common to all haemoglobins and their variants. The type of haemoglobin is determined by the protein part called globin. The polypeptide chains α, β, δ and γ make up normal human haemoglobins:

- haemoglobin A ..... = α 2 β 2
- haemoglobin A2 ..... = α 2 δ 2
- foetal haemoglobin F .....= α 2 γ 2

The α chain is common to all three haemoglobins.

The spatial structure of haemoglobin and other molecular properties (like those of all proteins) depend on the nature and sequence of the amino acids forming the chains.

Thus the substitution of certain amino acids by mutation is responsible for the formation of haemoglobin variants which have different surface charges and consequently different electrophoretic mobilities, which also depend on the pH and ionic strength of the buffer.

The resulting qualitative (or structural) abnormalities are called haemoglobinopathies. Reduced synthesis of one of the haemoglobin chains leads to quantitative (or regulatory) abnormalities, known as thalassaemias.

The assay is performed on the haemolysate from washed red blood cells. The haemoglobins are separated by electrophoresis on alkaline gels and the fractions are visualised by staining with amidoblack and then interpreted after drying **[13; 14]**.

**** Principle of the Sickle SCAN® test* [15]**

Sickle SCAN® is a rapid chromatographic qualitative lateral flow immunoassay for haemoglobins A, S and C for the identification of disorders associated with sickle cell disease. The test includes three indicators that detect the presence of these haemoglobins, enabling a rapid distinction to be made between normal, carrier and sickle cell samples **(Figure 1)**. Five microlitres of venous blood are placed in the buffered pre-treatment module to release the haemoglobin by lysis of the erythrocytes. Three drops of the treated sample are removed from the pre-treatment module and added to the sample inlet of the Sickle SCAN® cartridge. The sample interacts with the antibody-conjugated colorimetric nanoparticles and moves towards the capture zone. Results can be read within five minutes. The presence of haemoglobin variants A, S and C is indicated by lines in designated areas.

A total of four detection lines are possible, including haemoglobin variants A, S and C, plus a control line (which confirms that the test is working correctly). Samples containing two haemoglobin variants (such as composite heterozygotes) will have both variants detected **(Figure 1).**

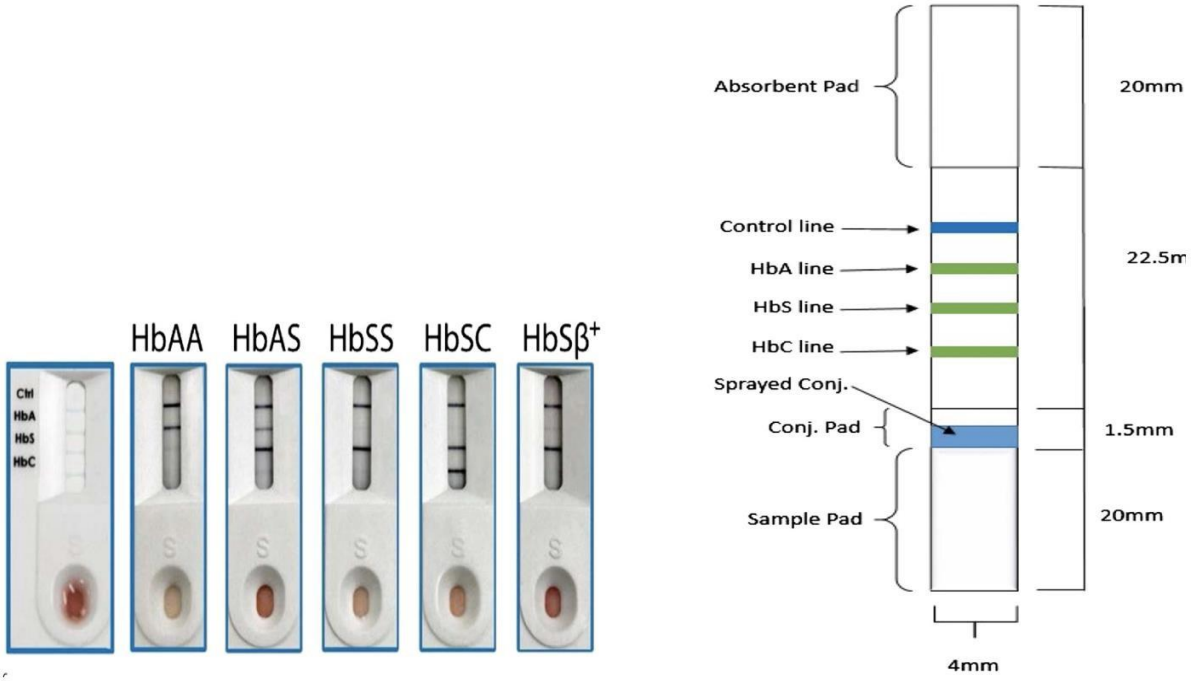

Figure 1: Schematic illustration of the design of the Sickle SCAN® strip with absorbent pad, control line, HbA line, HbS line, HbC line, conjugate pad, spray conjugates and sample pad.

II-6- Validation procedure

The validation procedure consisted of carrying out two types of test on the same samples from participants: the Sickle SCAN® rapid test (rapid test to be evaluated) compared with the conventional haemoglobin electrophoresis test carried out using a Sebia™ Hydrasis machine (reference method). In this study, the reference method used was the Sebia™ method, unlike other studies that used High Performance Liquid Chromatography (HPLC) or the Emmel test **[9; 16; 17]**. This choice is justified by the fact that we also wanted to compare the results of the Sickle SCAN test® with another reference method recognized worldwide in terms of haemoglobinopathy diagnosis.

II-7- Statistical analysis of the data

The performance of the Sickle S SCAN® test was evaluated using several statistical tests such as:

- *Confusion matrix*: The confusion matrix enabled us to visualize the correspondence between the results of Sickle SCAN® method and those of reference method.

- *Calculation of performance for each genotype*: Performance calculations for each genotype include sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy.

- *Kappa coefficient*: The Kappa coefficient was calculated to assess the concordance between the Sickle SCAN test and the reference method. A Kappa coefficient close to 1 indicates excellent agreement between the Sickle SCAN test® and the reference method, while a Kappa close to 0 indicates poor agreement.

- *Bland-Altman Plot*: We used the Bland-Altman Plot analysis to visualize the differences between the two methods for each subject and to detect any systematic bias between the two methods as well as the limits of agreement.

- *ROC analysis (Receiver Operating Characteristic)*: The ROC analysis was used to assess the ability of the Sickle SCAN® test to distinguish between different genotypes. The area under the curve (AUC) quantifies this ability, where an AUC of 1 indicates perfect performance and an AUC of 0.5 indicates performance at chance level **[18]**.

- *Chi2 test*: The Chi2 test was used to calculate p-values. A p-value < 0.05 suggests that the differences between the Sickle SCAN® method and the reference method are not due to chance;

i.e. these differences are statistically significant.

III- RESULTS

III-1- Patient profile and selected samples

A total of 300 patients who came to the clinical biochemistry and haemobiology unit of the Pasteur Institute of Côte d'Ivoire for biological analyses and who had given their informed consent were selected to participate in the study. Of these, 183 (61,0%) were female and 117 (39%) male. The age of the patients ranged from 1 month to 62 years, with a mean age of 22,8±16,96 years.

III-2- Prevalence of phenotypes observed

Analysis of the phenotypes detected by the two methods and their prevalence showed concordance between the results of Sickle SCAN® test and those of Sebia™ method (reference method). The AA2, ASA2, SA2, AC, CC and SC phenotypes were observed at prevalences of 40% (120/300), 29% (87/300), 8% (24/300), 18% (54/300), 2% (6/300) and 3% (9/300) respectively **(Table I)**.

Table I: Haemoglobin phenotype identified and frequencies according to techniques

Phenotypes	**Sickle SCAN® test (N=300)**		**Sebia™ method (N=300)**	
	patients (n)	Prevalences (%)	patients (n)	Prevalences (%)
AA2	120	40	120	40
ASA2	87	29	87	29
SA2	24	8	24	8
AC	54	18	54	18
CC	06	2	06	2
SC	09	3	09	3

N= total number of samples analyzed

n = number of each phenotype observed

III-3- Analysis of the performance of the Sickle SCAN® test

- Confusion matrix method:

The confusion matrix enabled us to visualize the correspondence between the results of the Sickle SCAN® test and those of the

reference method. The diagonal values represent cases where the two methods agree, while the other values indicate discrepancies (**Table II**). Our results from the confusion matrix method indicate that there is no discordance between the Sickle SCAN® test (test method) and the Sebia™ method (reference method) (**Table II**).

Table II: Confusion matrix for the Sickle SCAN® test performance analysis

Sickle SCAN® / Sebia™ method	AA2	AC	ASA2	C	SA2	SC
AA2	**40**	0	0	0	0	0
AC	0	**18**	0	0	0	0
ASA2	0	0	**29**	0	0	0
C	0	0	0	**2**	0	0
SA2	0	0	0	0	**8**	0
SC	0	0	0	0	0	**3**

- ***Sickle* SCAN® *test performance Calculation for the detection of each genotype:***

The performance calculated for each genotype includes sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy.

For the detection of the different genotypes, our results indicate a sensitivity of 100% and a specificity of 100% for both the Sickle SCAN® test (test method) and the Sebia™ method (reference method). (**Table III**).

Table III: Sickle SCAN® test performance calculation compared with the Sebia™ method

Genotypes	Sensitivitý	Specificitý	Positive Predictive Value (PPV)	Negative Predictive Value (NPV)	Overall accuracy
AA2	1	1	1	1	1
ASA2	1	1	1	1	1
SA2	1	1	1	1	1
AC	1	1	1	1	1
C	1	1	1	1	1
SC	1	1	1	1	1

- Kappa coefficient test:

The Kappa test showed a Kappa coefficient between the Sickle SCAN® test and the Sebia™ method equal to 1.

- ***Bland-Altman Plot:***

The Bland-Altman Plot shows the differences between the two methods for each subject. This graph can be used to detect systematic bias between the two methods and limits of agreement. This test revealed no bias between the Sickle SCAN® test and the Sebia ™ reference test (**Figure 1**).

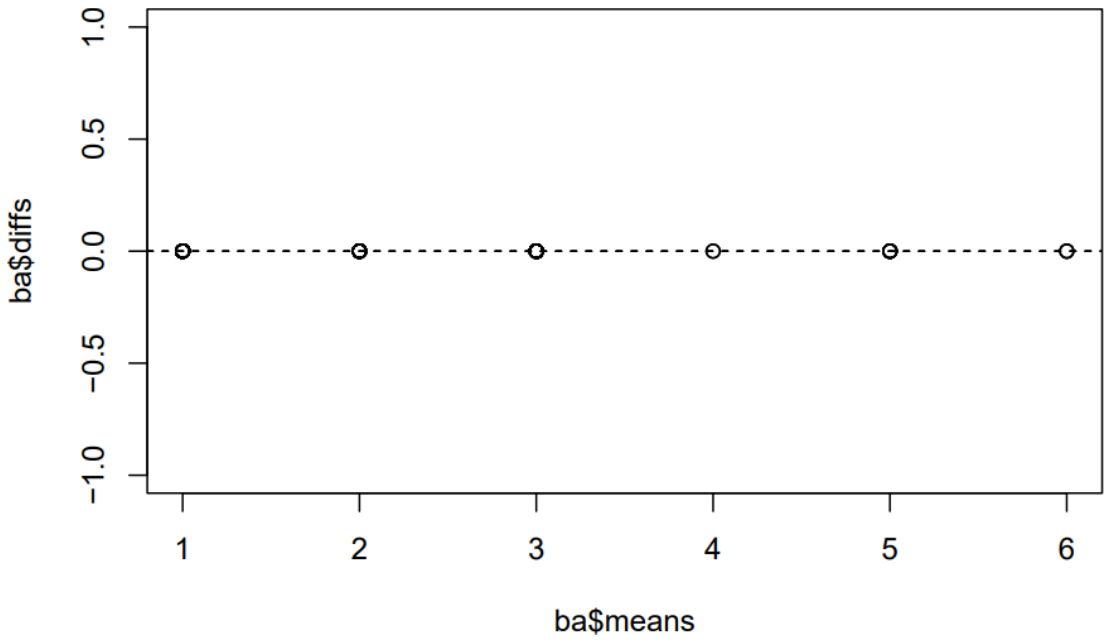

Figure 2: Results of the Bland-Altman Plot test

***- ROC analysis*:** The ROC analysis assesses the ability of the Sickle SCAN® test to distinguish between different genotypes. The area under the curve (AUC) quantifies this ability, where an AUC of 1 indicates perfect performance and an AUC of 0,5 indicates performance at chance level **(Figure 2).**

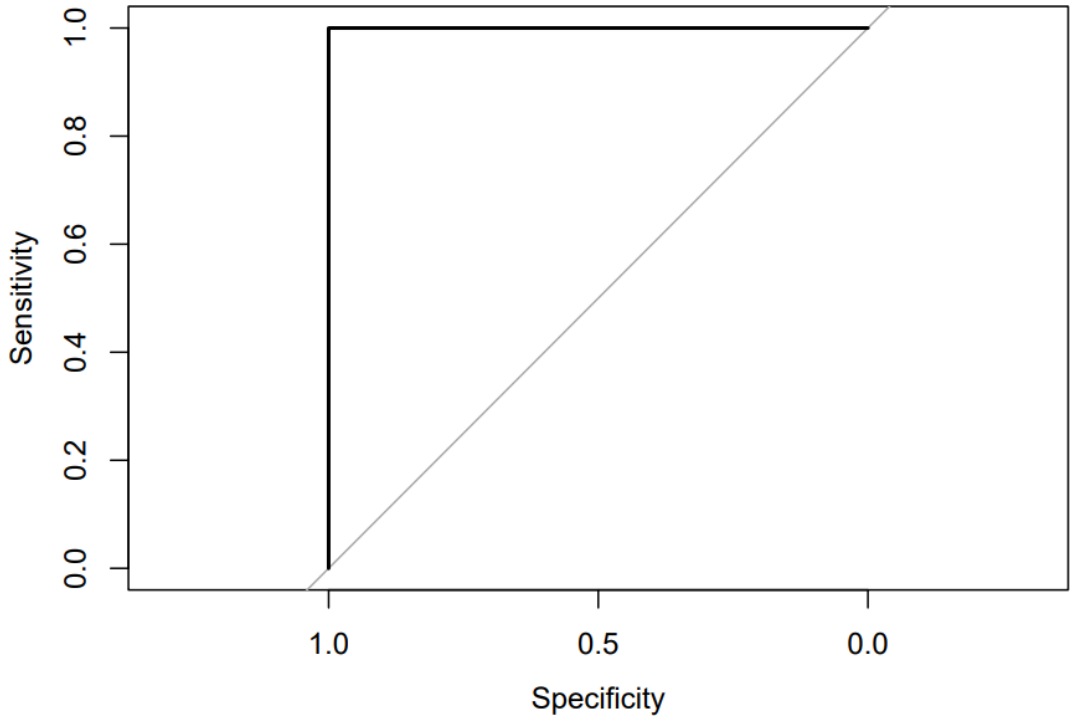

Figure 3: ROC curve for the Sickle SCAN® test

- Chi2 test: The Chi2 test was used to calculate p-values. A p-value <0,05 suggests that the differences between the Sickle SCAN® test and the Sebia™ reference method are not due to chance; in other words, these differences are statistically significant. Our results indicate that there is no significant difference between the results of the test method (Sickle SCAN®) and the reference method (Sebia™ method) (p> 0,05).

III-2- DISCUSSION

Sickle cell disease mortality and morbidity in Africa remain high despite initiatives to reduce the burden of the disease **[19; 20]**. This is because access to medical care is not uniform**.** Indeed, one of the first steps to ensuring equitable access to treatment and care for sickle cell disease, which is critical to reduce mortality and morbidity from the disease, is to screen communities effectively using accurate and inexpensive screening techniques.

In this study, we evaluated the performance of a rapid, low-cost test, the Sickle SCAN®, for the rapid detection of haemoglobins A, S and C in 300 patients. Several statistical tools were used to assess performance. The statistical tools provide a rigorous framework for evaluating the performance of a test and ensuring that the results are reliable, valid and relevant for decision-making. Analysis of the confusion matrix, the first statistical tool used, showed that there was no discrepancy between the results of the Sickle SCAN® test and those of the reference method (Sebia™ method). In fact, this statistical test, which has not been carried out in other previous studies, enabled us to show the close agreement between the results of these two techniques **[9; 16; 17]**. In addition, the results showed excellent sensitivity and specificity of the Sickle SCAN® test compared with the Sebia™ reference method for the detection of haemoglobins A, S and C. Performance analysis including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy showed values of 100% for each parameter. These indicators were used to assess the ability of the Sickle SCAN® test to correctly identify the genotype compared with the reference method. These results are in agreement with those of Steele et *al.* (2019) in the United States, Heavenlight et al. (2022) in Tanzania, Mungu et al. (2020) in the Democratic Republic of Congo (DRC) and Adjé et al. (2021) in Côte d'Ivoire, with specificities and sensitivities between 95 and 100% **[16; 17; 21; 22]**. Our results are also in line with those of Akueté et al. (2018) in Mali. Indeed, these authors used chromatography (HPLC) as a reference test and observed an estimated sensitivity and specificity of 100% for the sickle SCAN® test, regardless of haemoglobin phenotype. The same authors report that the Sickle SCAN® test perfectly predicts HbAS, HbAC, HbSS and HbSC phenotypes **[23].** The ROC analysis, which assesses the ability of the Sickle SCAN® test to distinguish between the different genotypes, showed perfect performance, with the area under the curve (AUC) equal to 1. Similarly, the Bland-Altman Plot test, which visualizes the differences between the two methods for each subject, revealed no systematic bias between the Sickle SCAN® test and the Sebia ™ reference test. As for the Kappa test, it showed a Kappa coefficient of 1 between the Sickle SCAN® test and the Sebia ™ method, indicating excellent agreement between the two methods. Finally, the Chi2 test was used to calculate a p-value of less than 0,05, suggesting that the concordances between the Sickle SCAN® test and the Sebia™ reference method are not due to chance.

Our study has some limitations in that no cases of S/β thalassaemia were detected; but the principle of the Sickle SCAN® test is that minor haemoglobin F and A2 fractions are determined as well as body iron reserve levels and sometimes even genetic family studies **[24].** Thus, we have not evaluated the accuracy of the test in the neonatal period although evaluating the accuracy of the Sickle SCAN® test on fetal haemoglobin forms is an interesting prospect.

CONCLUSION

In sub-Saharan Africa, it is estimated that around 90% of children with sickle cell anaemia die undiagnosed before the age of 5. This makes the disease one of the main causes of infant mortality in the region. Hence the need for an accessible, simple, rapid and reliable diagnostic technique in rural areas, which are generally a long way from major health centers with dedicated equipment. The analysis showed that the Sickle SCAN® test was highly consistent with the Sebia™ reference method, with robust results for the detection of the different genotypes. The Kappa coefficient and the ROC curve confirmed the validitý of the Sickle SCAN® test as a reliable tool for rapid screening for sickle cell disease. This test could therefore represent a credible alternative for sickle cell disease diagnosis.

ETHICAL CONSIDERATIONS

The study protocol was approved by the Ministry of Health's ethics committee. Confidentiality was maintained throughout the study. The informed consent of the study participants was obtained prior to their inclusion in the study. Referral of cases of major sickle cell syndromes to the haematology department of the Cocody Hospital Center (Abidjan, Côte d'Ivoire) was recommended, as well as genetic counselling for haemoglobin abnormalities.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest in this work.

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facilities of the Biochemistry Laboratory of the Pasteur Institute of Côte d'Ivoire to carry out haemoglobin electrophoresis using a Sebia™ Hydrasis machine at the clinical biochemistry and haemobiology unit. They would also like to thank Mr BAMBA Zah, Director of the Phalangis company, for his material and logistical support.

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