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ORIGINAL ARTICLE

Performance evaluation of the Hemotype SC™ rapid test for the diagnosis of sickle cell disease

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Abstract

Introduction: The use of Rapid Orientation Diagnostic Tests ("RODTs") could play an important role in the mass screening of certain diseases. It saves a great deal of time while managing the flow of samples and the difficulties associated with their storage. This study aimed to identify the impact of the delay between sampling and analysis on the analytical performance of the Hemotype RODT SC™.

Methods: This was a descriptive cross-sectional study conducted over one month. The Immunology-Hematology Department of the CHU de Cocody served as the study setting. The study population consisted of sickle cell patients and hemoglobin C carriers of all ages and sexes. We performed hemoglobin electrophoresis on all samples on the day of collection (D0) and the Hemotype SC™ rapid test on D0, D2, D4, and D6. Sensitivity and specificity were used to determine analytical performance, and the chi2 test was used to compare qualitative variables.

Results: The study involved 102 patients with a median age of 16 years (IQR: 9-24). Half of the participants (56.96%) were SSFA2 homozygous sickle cell disease major. On average, 87.1% of RODT results were valid from D0 to D6. At D0, the RODT had a sensitivity of 75% and a specificity of 81.40% for Hb A; Hb S had a sensitivity of 79.80% and a specificity of 100%; Hb C had a sensitivity of 35.71% and a specificity of 98.65%. The sensitivity of Hb C and the specificity of Hb A had increased statistically significantly over time. Cross-referencing the RODT results from D0 to D6 with those of electrophoresis showed a concordance of 86.95% for the AA phenotype, 79.52% for AS; 88.52% for SS; and 95.45% for SC.

Conclusion: The HemoTypeSC™ test, with a sensitivity of 75% and specificity of 98% is suitable in certain areas where access to electrophoresis is limited. This performance is not impaired on samples stored for 6 days.

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Introduction

Sickle cell anemia is a constitutional hemoglobinosis with autosomal recessive inheritance. It is characterized by a point mutation in codon 6 of the β-chain gene, resulting in the replacement of glutamic acid by valine. This leads to an abnormal, poorly soluble hemoglobin (Hb) (Hb S) [1]. It is the most common genetic disease in the world [2]. According to the WHO, the disease affects more than 50 million subjects (homozygotes), and there are 250 million heterozygote carriers worldwide [1]. More than 330,000 children are born with this disease every year, and 80% of them die before their fifth birthday if they are not medically monitored [1]. Sickle cell disease is most prevalent in sub-Saharan Africa and is now a public health problem in most black African countries. In Central and West Africa, 20 to 40% of subjects carry the sickle cell trait [1]. In Côte d'Ivoire, the prevalence of this hemoglobinopathy is 12%, of which 2% is major [3].

Hb C prevalence is over 15% in West Africa [4]its diagnosis and genetic counselling are important to prevent inheritance with other haemoglobinopathies. Little is known about its contemporary distribution and the number of newborns affected. We assembled a global database of population surveys. We then used a Bayesian geostatistical model to create maps of HbC frequency across Africa and paired our predictions with high-resolution demographics to calculate heterozygous (AC. Clinically, it is asymptomatic in heterozygotes. When Hb C is associated with Hb S, it presents as SC, which belongs to the group of major sickle cell syndromes. In Côte d'Ivoire, sickle cell disease is diagnosed using tests such as hemoglobin electrophoresis on agarose gel and hemoglobin electrophoresis at alkaline or acid pH coupled with the Emmel test. This test requires an appropriate analysis laboratory, suitable equipment, and qualified personnel [5]. These particular conditions make it difficult for our populations to access, and even impossible for those in remote areas, hence the interest in using a rapid diagnostic test such as the HemoType SC™ test. The WHO recommends the use of simple, inexpensive rapid orientation tests (RODT) in resource-limited countries, which can be used in laboratories in peripheral centers [6] At the same time, these tests must have comparable performance to the reference methods, i.e. electrophoretic techniques. To achieve this, they must be evaluated in the areas where they are to be used [3]. The SC HemoType SC™ rapid test is a simple, inexpensive test that could be used for systematic screening for sickle cell disease in Côte d'Ivoire, and even in the most remote areas. It requires no special equipment, electricity, or refrigerator for storage. The test has been designed so that it can be carried out at the sampling site without the need to transport samples elsewhere, but in screening campaigns, it is sometimes not possible to carry out tests at the same time. This study aimed to identify the impact of

the delay between sampling and analysis on the analytical performance of the HemoType SC™ RODT

Material and methods:

Study site and population:

Participants were recruited at the Immunohematology Department of the University Hospital of Cocody, Abidjan. We conducted a descriptive cross-sectional study over one month from November 15 to December 15, 2021. The study population consisted of sickle-cell patients and Hb C carriers of any age and sex attending the Immunohematology Department at the University Hospital of Cocody.

Inclusion criteria

We included in our study all major sickle cell patients with compliant specimens; major sickle cell patients who had not been transfused in the three months before the study; and adults and parents of sickle cell children who had given informed consent.

Study process

After obtaining the participants' consent, we proceeded to fill in the survey forms. Each participant had a consultation with a hematologist. A venous sample was taken and collected in a tube containing an anticoagulant, ethylene diamine tetra-acetate (EDTA) to perform the HemoTypeSC™ rapid test and hemoglobin electrophoresis. Blood samples were transported to the laboratory. On the day of collection (D0) for each participant we performed the HemoTypeSC™ test and hemoglobin electrophoresis on a cellulose acetate plate which constituted our reference test. Three (3) aliquots of each sample were taken and stored in the refrigerator at +2 to +8°C for testing with HemoTypeSC™, at D2, D4, and D6. To determine the participants' hemoglobin phenotype, we systematically used the HemoTypeSC™ rapid strip test, which is a rapid diagnostic orientation test for hemoglobinosis S and C. It is a competitive immuno-chromatographic test using monoclonal antibodies to detect the presence of Hb A, S, and C in a hemolysate. The deposition zone of the test contains red colloidal gold particles conjugated to antigens similar to Hb A, S, and C, and to mouse immunoglobulin (IgG). Using a dropper pipette, add six (6) drops of water to a hemolysis tube; draw 1-2 microliters of blood using a sampling device; bring the blood into contact with the water, inserting the sampling device into the hemolysis tube, and shake to mix until the water turns pink or light red; insert the test strip into the hemolysis tube (arrows pointing downwards); wait 10 minutes, then remove the test strip from the hemolysis tube and read the result. Interpretation of the test is based on the fact that the presence of a line indicates the absence of the corresponding Hb fraction; the absence of a line indicates the presence of that Hb fraction; if the control line is absent, or all lines are present, the result is invalid. Electrophoresis of hemoglobin on cellulose acetate at alkaline pH was our reference test. This method separates electrically charged particles by differential migration under the action of an electric field.

Data analysis:

The characteristics of study participants were described using basic descriptive statistics. Diagnostic tests were used to test our hypothesis that the two screening methods (cellulose acetate electrophoresis and HemoTypeSC™,) were not significantly different using specificity, and sensitivity in diagnosing individuals with different Hb phenotypes (AA, AS, SC, and SS). Receiver operating curves (ROC) were calculated with their corresponding areas under curves

(AUC) to compare the performance of each diagnostic method. Stata version 16 software was used for data analysis, and chi-2 was used to compare variables, and p-values <0.05 were considered significant.

Results

In the course of this study, we registered 107 patients, of whom 3 had been transfused in the three months preceding the study and 2 had not been sampled. A total of 102 patients took part in the study, i.e. 95.32% (figure 1). Concerning socio-demographic data (Table 1), the median age of participants was 16 years (IQR: 9-24), with extremes ranging from 1 to 46 years. More than half the participants had at least a primary school education. The values summarized in Table 2 show the RODT results at D0 compared with the reference test, hemoglobin electrophoresis. The test correctly identified 50/53 with SS phenotype. The test identified 18 patients with AS phenotypes, unlike electrophoresis, which is due to the test's inability to detect fetal hemoglobin (F); SAFA2 phenotypes on electrophoresis are identified as AS by the RODT. Of the 27 SC phenotypes identified on electrophoresis, the test identified only 11, with a high percentage of invalid tests (12.75%). The results of the RODT of hemoglobin phenotypes from D0 to D6 and the patients' electrophoresis results are summarized in Table 3.

We cross-referenced the RODT results from D0 to D6 with those of hemoglobin electrophoresis to determine the concordance between the patients' hemoglobin phenotypes. These results showed a concordance of 86.95% for the AA phenotype, 79.52% for the AS phenotype; 88.52% for the SS phenotype; and 95.45% for the SC phenotype.

At D0 we found a sensitivity of 75% for Hb A and a specificity of 81.40% (table 4). The sensitivity of the test did not vary with the time between the time of sampling and the days of testing (p-value < 0.05), but the specificity of the RODT increased statistically significantly (p-value < 0.05). For Hb S, the sensitivity of the test was 79.80% at D0. The sensitivity of the RODT did not vary between the time of sampling and the day of testing (p-value < 0.05). Specificity was 100%, i.e. the test was able to confirm the absence of Hb S in subjects who did not have it. The sensitivity of the test was 35.71% for Hb C. The sensitivity of the RODT increased statistically significantly from the time of sampling to D6 (p-value < 0.05). Specificity was 98.65%.

Areas under the curve (AUC) showed the level of concordance for the different phenotypes between cellulose acetate electrophoresis and HemoTypeSC™ from D0 to D6 (Figure 2). The AUCs are respectively (D0=0.869; D2=0.892; D4=0.809; D6=0.887). These figures showed a lower level of concordance with electrophoresis.

Discussion

Sickle cell anemia is a serious hereditary genetic disorder that is a major public health problem in black Africa [7]. Management of this condition could be more effective and less costly if diagnosis were made at an early stage. However, screening is often delayed until clinical signs appear, partly because the method currently used (Hb electrophoresis) requires extensive equipment. In sub-Saharan Africa, sickle cell disease is often identified late. This hurts morbidity and mortality [7].

The rapid test being evaluated in this study is HemoTypesSC™, a promising new method that could then enable everyone to know their hemoglobin profile and thus be directed as early as possible toward therapeutic management. Because of the limitations of some screening tests, the WHO recommends that tests be evaluated before they are used in a given region [6]. The method used as a reference is Hb electrophoresis on a cellulose acetate plate. We considered its specificity to be absolute by definition. This reference is

valid for comparison with the RODT.

The SS homozygous form affected more than half the participants in our study. Similar results were observed in an earlier study carried out in 2021 in Nigeria [8]. This was a competitive immuno-chromatographic test using monoclonal antibodies to detect the presence of Hb A, S, and C in hemolysate. HemoTypeSC™ cannot differentiate between SSFA2 subjects and SFA2(β0 thalasso-sickle cell disease form) nor between SAFA2 (β+thalasso-sickle cell disease) and AS because HemoTypeSC™ fails to highlight Hb F.

We found a concordance of 86.9% for the AA phenotype, 79.5% for the AS phenotype; 88.5% for the SS phenotype; and 95.4% for the SC phenotype. These results show that the interval between the day of sampling and the day of testing does not influence the validity of the results.

For HbA, the HémoTypeSC™ test had a sensitivity of 75% and a specificity of 81.40% at D0. Studies in Côte d'Ivoire in 2020 [9] and Nigeria in 2019 [10] reported sensitivities of 100%. This difference can be explained on the one hand by the size of the sample [9,11] and by the difference in reference tests for the multicenter study (Ghana-Martinique-USA), which used either capillary electrophoresis, isoelectric focusing or high-performance liquid chromatography. There was no significant variation in the sensitivity of the test for the detection of Hb A from D0 to D6. However, specificity had increased statistically significantly (p-value < 0.05).

For the detection of Hb S, we found a sensitivity of 79.80% and a specificity of 100%. A feasibility and acceptability study carried out in Lagos, Nigeria [12] reported a sensitivity and specificity of 100% with HemoTypeSC™. This difference could be explained by the fact that the tests were performed at the point of intervention, i.e. in the delivery room, and could be more sensitive. From D0 to D6 there was no variation in sensitivity and specificity (p-value < 0.05).

For Hb C, we found a sensitivity of 35.71% and a specificity of 98.65% at D0. This sensitivity increased statistically significantly from D0 to D6 (p-value < 0.05). In India [13] and the frequency of the sickle cell gene is very high in the remote tribal areas where facilities are generally limited. Therefore, a rapid and affordable point-of-care test for sickle cell disease is needed.\n \n \n Methods\n The diagnostic accuracy of HemoTypeSC was evaluated against automated high-performance liquid chromatography (HPLC and Steele et al [11] among participants from Ghana, France, and the USA, the prevalences of hemoglobin AC and SC were low, while Nankanja et al. [14] in Uganda, reported no HbC.

In short, for Hb A, S, and C, we found low sensitivities and specificities in contrast to those found by other studies carried out in Africa [9,15] with sensitivities and specificities over 99%.

In our study, we found on average 13.9% of discordant results in the same direction as Malay et al in 2020 [13]and the frequency of the sickle cell gene is very high in the remote tribal areas where facilities are generally limited. Therefore, a rapid and affordable point-of-care test for sickle cell disease is needed. \n \n \n Methods\n The diagnostic accuracy of HemoTypeSC was evaluated against automated high-performance liquid chromatography (HPLC (28 cases). Another possible reason for the divergent results of our study could be the high percentage of beta-thalassemic forms with the presence of hemoglobin F. As in Tanzania [16] where all children were tested on days 1 and 2 of life, when HbF was at its peak in other studies [8] HemoTypeSC™ showed high accuracy and concordance compared to PCR and high-performance liquid chromatography.

Our study encountered several constraints. The reference test was not performed on

all days (D2 D4 and D6) these results were compared only to the D0 electrophoresis. At D0 we already had several discordant and invalid HemoTypeSC™ tests it would be suitable to have a third discriminatory test to remove the discordance. As the sample size was small, we did not determine the predictive values.

Conclusion

Our study shows that HemoType is useful and could be appropriate for mass screening and prevalence studies. It has good specificity, even if it sometimes shows discrepancies with the reference test in the identification of Hb A, S, and C. The time between sampling and the day of testing did not influence the results. A re-evaluation study of the test would be desirable, with a large sample size to determine predictive values. PCR and high-performance liquid chromatography would be ideal for the gold standard.

However, this test has limitations as it does not detect Hb F and the reading is inverted, as the band that appears represents the hemoglobin fraction, which is absent. It could be combined with Hb electrophoresis.

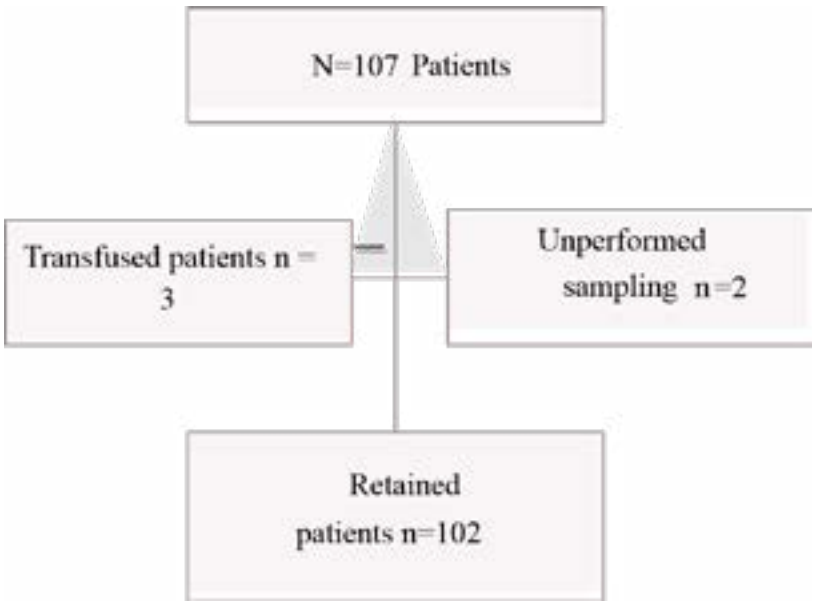

Figure 1: Study population summary diagram

Table 1: Breakdown of results by socio-demographic data

Characteristics	N = 102 (%)
Age (year) Median [IQR]	16 [9 - 24]
≤15	51 (50.0)
16-30	33 (33.0)
>30	18 (18.0)
Sex, n (%)	
Female	62 (60.7)
Male	40 (40.0)
Education level, n (%)	

Did not attend school	5 (5.0)
Primary	42 (41.1)
Secondary	32 (32.0)
Higher	23 (23.0)
Profession, n (%)	
Pupil	60 (58.8)
Student	16 (16.0)
Other	26 (26.0)

*IQR: interquartile range

Table 2 : Comparaison entre les résultats de l'électrophorèse et le RODT à J0

Phenotypes	RODT **J0**	**Electrophoresis Hb**
	n(%)	**n(%)**
SS	50 (49,0)	53 (56,8)
AS	18 (17,6)	2 (13,7)
SC	11 (10,7)	27 (26,4)
CC	0 (0)	1 (0,98)
AA	10(9,8)	2 (1,9)
Invalid	13(12,7)	0 (0)
SFA2	0(0)	5 (4,9)
SAFA2	0(0)	12 (11,7)

Table 3: RODT results according to the interval between sampling and testing

	Test results				
Phenotype	J0 n (%)	J2 n (%)	J4 n (%)	J6 n (%)	Electrophoresis results n (%)
AA	10(9.8)	2(1,9)	2 (1,9)	2 (1,9)	2 (1,9)
AS	18 (17,6)	11 (10,8)	9 (8,8)	19(18,6)	14 (13,7)
SS	50(49,0)	59 (57,8)	58(56,8)	48 (47,0)	58 (56,8)
CC	0(0)	0(0)	1(0,9)	1(0,9)	1(0,9)
SC	11 (10,7)	16(15,6)	16(15,6)	22 (21,5)	27 (26,4)
Invalid	13 (12,5)	14 (13,7)	16(15,6)	10(9,8)	-

Table 4: Intrinsic characteristics of hemoglobin variants

		D0	D2	D4	D6
	Sensitivity	75%	68,7%	56,2%	81,2%
	[CI]	[*66,60-83,40*]	[*59,75-77,75*]	[*46,62-65,88*]	[*73,68-88,82*]
Hb A	*p-value*	-	*0,69*	*0,26*	*0,66*
	Specificity	81,40%	97,67%	97,26%	90,70%
	[CI]	[*73,84-88,95*]	[*94,75-100*]	[*94,75-100*]	[*85,06-96,33*]
	p-value	-	*0,005*	*0,005*	*0,07*
	Sensitivity	79,8%	86,8%	98,8%	83,8%
	[CI]	[72*,01-87,59*]	[80*,31-93,42*]	[96*,57-100*]	[76*,69-90,98*]
Hb S	*p-value*	-	*0,18*	*0,59*	*0,05*
	Specificity	100%	100%	100%	100%
	[CI]	-	-	-	-
	p-value	-	-	-	-
	Sensitivity	35,7%	57,1%	60,7%	82,1%
	[CI]	[26*,42-45,01*]	[47*,54-66,75*]	[51*,24-70,19*]	[74*,71-89,58*]
Hb C	*p-value*	-	*0,18*	*0,59*	*0,0004*
	Specificity	98,65%	100%	100%	100%
	[CI]	-	-	-	-
	p-value	-	*0,31*	*0,31*	*0,31*

*IC: Confidence interval

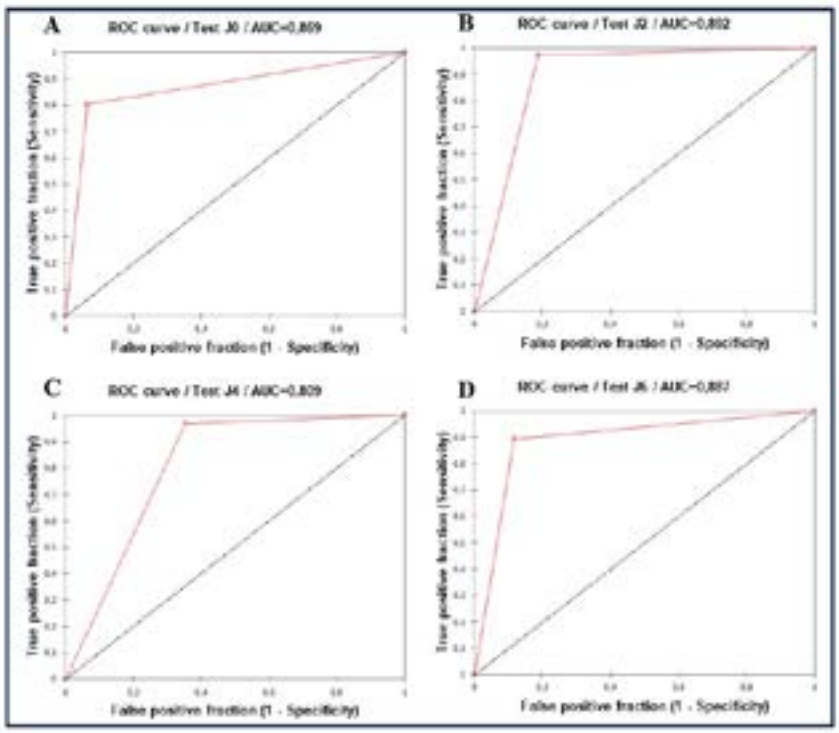

Figure 2: ROC curve for HemoTypeSC™ versus cellulose acetate electrophoresis**.**

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