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Validation of the sickle scan technique used to diagnose sickle cell disease

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ABSTRACT

Introduction
Sickle cell disease is a multifactorial haemoglobinopathy that causes various abnormalities in affected individuals. Several diagnostic methods exist, but most require sophisticated techniques that are not available in Congolese diagnostic centres involved in this activity. Considering the number of affected individuals in Africa, particularly in the Democratic Republic of Congo (The Democratic Republic of the Congo) (50 million people affected), and the precarious financial situation of our countries, it is essential to develop low-cost techniques to facilitate access to screening for our populations.

Purpose
This study aimed to validate the rapid "Sickle SCAN" method, manufactured by ZENTECH, as a tool for rapid diagnostic testing.

Methods
We assessed a single validation criterion: selectivity. This was done using samples with known haemoglobin status. The results of the Sickle SCAN test obtained from 290 subjects were compared with those obtained through isoelectric focusing, the reference method, using the Chi-square test. The duration for trait appearance was also evaluated.

Results
The Sickle SCAN test demonstrated 100% selectivity. Statistical analysis using the Chi-square test confirmed that the results from isoelectric focusing were identical to those obtained using the Sickle SCAN method. This test can also be applied to samples collected on blotting paper. The interpretation time should not exceed 2 minutes, contrary to the manufacturer's claims.

Conclusion
The Sickle SCAN rapid screening test, manufactured by ZENTECH, produced satisfactory results and is suitable for use by the Congolese population to screen for sickle cell disease. This test has the advantages of being accessible, available, and easy to use.

INTRODUCTION

Sickle cell disease is a haemoglobinopathy in which glutamic acid at position 6 on the ß chain is replaced by valine (Tchoteu, 1973; Sow, 2006). This autosomal mutation produces abnormal haemoglobin, known as haemoglobin S (HbS), which causes complications affecting several organs (Nacoulma et al., 2006; Kpowbie, 2001).

Sickle cell disease is the most widespread genotypic disorder globally, affecting populations in the Black race, the Mediterranean basin, the Middle East, and Southeast Asia. The disease is prevalent throughout Africa, with several million carriers. In some peri-equatorial countries, 30% to 40% of the population is affected, including the Democratic Republic of Congo, where more than 50 million people are carriers of the sickle cell trait (Dreyfus et al., 1984; Gentilini et al., 1983; Naik & Haywood, 2015; Key & Derebail, 2010; World Health Organization [WHO], 2010).

The disease is characterised by the presence of sickle-shaped cells in the blood (Chalacheva et al., 2019; Uhelski et al., 2019). It is accompanied by several acute clinical manifestations, such as vaso-occlusive crises, acute thoracic syndrome, stroke, and chronic complications such as haemolytic anaemia, infections, hip osteonecrosis, leg ulcers, nephropathy, and growth retardation (Habibi et al., 2015; Godeau, 2004; Yawn et al., 2014). Given its prevalence and complications, sickle cell disease constitutes a significant public health problem, prompting many countries to implement systematic screening programmes (Hubert et al., 2003).

Several methods are used to diagnose sickle cell disease, including electrophoretic methods with various variants, chromatographic methods such as HPLC, and the Emmel method. This study focused on validating a more practical and rapid method to reduce costs associated with reagents and materials required by the aforementioned techniques.
Before using this technique on a large scale in the Democratic Republic of the Congo, it was necessary to validate it. Currently, no analytical method can be routinely used without validation. Validation ensures the reliability of results obtained during routine use. Regulatory guidelines, such as Good Manufacturing Practice (GMP), Good Laboratory Practice (GLP), and documents from organisations like ISO, ICH, and FDA, require that all assay procedures meet specific acceptability criteria (Hubert et al., 2008). Since the Sickle SCAN method was recently introduced in the Democratic Republic of the Congo, validation was deemed essential to ensure reliable results.

Sickle cell disease is a significant public health issue in The Democratic Republic of the Congo due to its high prevalence. In hospitals across the country, most laboratories rely on electrophoretic methods, which are often costly and dependent on inputs that, when unavailable, render the method unusable. Additionally, electricity outages frequently delay the delivery of results.
The introduction of a rapid screening test for sickle cell anaemia in the Democratic Republic of the Congo, once validated, could address these challenges. The test's simplicity allows it to be used even in the absence of electricity, making it applicable throughout the country, including in settings outside laboratories. The Biochemistry-Haematology Laboratory of the Faculty of Pharmaceutical Sciences at the University of Kinshasa served as the setting for this study.

METHODS

Inclusion Criteria

This study included all blood samples suspected and tested positive for falciformation using the reference technique for comparison with the method under study.

Exclusion Criteria

Blood samples that did not meet the inclusion criteria were excluded from the study.

Specimens

Blood specimens were provided by the *Centre Mixte d'Anémie SS* (CMASS) located in the Kalamu commune of Kinshasa, Democratic Republic of the Congo. These included two types of specimens: 30 samples with known haemoglobin status (10 AA, 10 AS, and 10 SS) and 290 samples with unknown haemoglobin status.

The test under evaluation was the TDR Sickle SCAN (a rapid qualitative test), manufactured by ZENTECH, a Belgian company. The test is based on immunological

reactions for identifying haemoglobins A, S, and C. It was evaluated using both EDTA and blotting paper samples.

Validation

Since the method is qualitative, validation involved assessing selectivity and comparing results with those from a reference sickle cell screening method, "isoelectric focusing" (Hubert et al., 2003, 2008; MLHE, 2007). The duration of line appearance was also assessed.

Statistical Analysis

The Chi-square statistical test was used for statistical comparisons (Schwartz, 1963) due to the dichotomisation of collected data.

RESULTS

Selectivity Assessment

We worked with 30 samples, comprising 10 AA, 10 AS, and 10 SS. The results are presented in **Table 1**.

Table 1:
Method Selectivity

N°	AA	AS	SS
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
9	+	+	+
10	+	+	+

Legend: +: Results confirmation; -: Results not confirmed

Statistical Analysis

The data obtained from the diagnostic tests were dichotomized, and the chi-square test was applied to evaluate whether the results were consistent across the two devices tested. Statistical analyses were conducted using Excel and R Commander software. A difference was considered significant if the *p*-value was less than 0.05 (α), with a 95% confidence interval.

Comparison of Isoelectric Focusing Results with Sickle SCAN Results

Table 2:
Comparison of Isoelectric Focusing Results and Sickle SCAN Results

Statut d'hémoglobine	Methods	
	Isoelectric focusing	**Sickle Scan**
AA	119/290	119/290
AS	113/290	113/290
SS	58/290	58/290
X-squared	0	
Q- X-squared (α= 0,05)	5,991 (df = 2), p-value = 1	

This **Table** demonstrates that the results obtained through isoelectric focusing are identical to those obtained using the Sickle SCAN method. Statistical analysis using the chi-square test of homogeneity confirmed this equivalence rather than relying on visual observation.

Sickle SCAN and Blotting Papers

Samples with known haemoglobin statuses, fixed on blotting papers, were analysed using the Sickle SCAN method. The results are presented in **Table 3**.

Table 3:
Sickle SCAN Results on Blotting Papers

N°	AA	AS	SS
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+

Legend: +: Results confirmation; -: Results not confirmed

Evaluation of Test Duration

The manufacturer recommends evaluating results within five minutes. We assessed the test duration, and the results are presented in **Table 4**.

Table 4:
Test Duration

	Test 1	Test 2	Test 3
Sample AA	1 min 56 sec	1min 56 sec	1min 57 sec
Sample AS	1min 58 sec	1 min 56 sec	1min 59 sec
Sample SS	1min 57 sec	1 min 57 sec	1 min 56 sec

This **Table** indicates that the duration of the test does not exceed two minutes.

DISCUSSION

Our study focused on validating a rapid screening test for sickle cell disease, known as Sickle SCAN, manufactured by ZENTECH. This test had recently been introduced in the Democratic Republic of the Congo market. Our team deemed it necessary to validate the method before adopting it as an alternative to the existing screening or diagnostic methods for sickle cell disease.

To meet this objective, we assessed one key validation criterion for analytical methods: selectivity, given the qualitative nature of this method. Subsequently, we compared the results of isoelectric focusing, used as the reference method in our laboratory, with those of the Sickle SCAN method. Blood specimens were collected in EDTA tubes for this comparison. Additionally, we explored the potential applicability of the Sickle SCAN method to blood specimens collected on blotting paper.

Selectivity Assessment

Selectivity was evaluated on 30 samples of known hemoglobin status. The results, presented in Table I, demonstrated that the Sickle SCAN method is selective. It confirmed the results of all 30 samples of known hemoglobin status, aligning with the findings of previous studies (Hubert et al., 2003; Hubert et al., 2008). No interference was observed, as there were no additional bands suggesting artifacts. However, after the recommended 5-minute result reading period, we noticed that hemoglobin degradation under our working conditions caused additional bands to appear. Based on this observation, we recommend reading the results within 2 minutes instead of the manufacturer's suggested 5 minutes.

Comparison with Isoelectric Focusing

The results of isoelectric focusing on 290 samples were compared with those of the Sickle SCAN method using the Chi-square statistical test (Schwartz, 1963). Statistical analysis revealed no significant differences between the two methods. The Sickle SCAN method is, therefore, a valid alternative to isoelectric focusing under the specified conditions.

Sensitivity and Specificity

Our study confirms that the Sickle SCAN method is both sensitive and specific, suitable for screening and diagnosis. Previous research corroborates our findings. For instance, a study on 104 newborn samples compared the reference chromatographic method with the Sickle SCAN rapid diagnostic test (Nguyen-Khoa et al., 2018). Another study assessed the diagnostic accuracy of the method, confirming its reliability for clinical validation using capillary blood from individuals over one year of age (Kanter et al., 2015). The Sickle SCAN method effectively detects hemoglobin A, S, and C, providing results visible to the naked eye.

Application to Blotting Paper Samples

The applicability of the Sickle SCAN method to blood specimens collected on blotting paper was confirmed in our study, as shown in Table III. Previous research also supports the method's reliability on both whole blood and blotting paper samples (Nguyen-Khoa et al., 2018; Kanter et al., 2015).

Evaluation of Test Duration

The manufacturer recommends evaluating results within 5 minutes. However, as shown in Table IV, we found that the results could be reliably read within 2 minutes. Beyond this time, artifact bands appeared, potentially altering the interpretation of results (e.g., misclassifying AA samples as AS). This phenomenon was also observed in cassettes stored for extended periods. We hypothesize that the artifact bands result from hemoglobin degradation, potentially influenced by environmental factors such as climate.

Relevance to the Local Context

The Sickle SCAN method is well-suited to the working conditions in the Democratic Republic of the Congo, which are often characterised by frequent power outages. Its ease of use allows for sickle cell disease screening in remote or resource-limited settings, reducing the number of births of children with sickle cell disease. The Sickle SCAN™ test has significant potential to improve diagnosis and treatment globally while enhancing genetic counselling at the point of care (Julie Kanter et al., 2015).

CONCLUSION

The Sickle SCAN test is a sensitive, specific, and reliable method for diagnosing hemoglobin A, S, and C. It is fast, affordable, and easy to use, making it suitable for both diagnosis and screening in diverse settings, including those with limited resources or frequent power interruptions. The method offers advantages such as speed, low cost, and ease of interpretation, making it a valuable addition to diagnostic laboratories. We recommend its widespread adoption across the country.

Ethical Approval: Not applicable

Conflicts of Interest: None declared.

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