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# Newborn screening for sickle cell disease in Angola: Implementation challenges and emerging data on hemoglobinopathy prevalence

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ABSTRACT

Sickle Cell Disease (SCD) is an autosomal recessive disorder with a substantial global burden. Despite its particularly high incidence in sub-Saharan Africa, early diagnosis remains limited in many countries. The objective of this study was to implement a newborn screening programme for SCD in one of the largest maternity hospitals in Angola and to support the subsequent pediatric follow-up of the affected children.

Between June 2023 and December 2024, all children born in or attending the main hospital for vaccination were screened after parental or guardian consent. Blood was collected by heel-prick onto filter paper, and haemoglobin electrophoresis was performed by isoelectric focusing. Samples identified as HbSS were confirmed by PCR-RFLP, and atypical electrophoretic patterns were further analysed by DNA sequencing.

In a total of 13,256 samples analysed the prevalence of HbSS was 1.38% ($n = 183$), and 20.31% ($n = 2692$) were HbAS. Other variants identified ($n = 44$) included HbE, HbC, and α-globin gene alterations. Of the infants diagnosed with SCD, 106 (58%) families were successfully contacted, but only 76 (42%) children initiated regular medical follow-up and prophylactic treatment (penicillin, multivitamins, and vaccinations. 30 families (28%) declined treatment and just one declined follow-up.

These findings confirm the high prevalence of SCD in Angola and demonstrate the capacity of newborn screening programmes in reducing early morbidity and mortality. However, the substantial proportion of families refusing follow-up highlights the need for strengthened community health education to improve understanding of SCD.

## 1. Introduction

Sickle cell disease (SCD) is an autosomal recessive inherited blood disorder caused by a mutation in the gene encoding the β-globin chain of haemoglobin. SCD is life-limiting disorder associated with high premature death and end organ damage [1].

Although the disease burden is especially high in sub-Saharan Africa, access to early diagnosis remains limited and no country has established a national screening program yet across the region. In high-income developed countries, the introduction of universal newborn screening and prompt intervention has virtually eliminated early childhood mortality associated with sickle cell disease [2] Conversely, in low-income developing regions, the absence of early detection and adequate medical care results in 50% to 80% children with SCD dying before the age of five [3]. Multiple studies have demonstrated that implementing newborn screening programs, combined with specialized clinical follow-up, can increase survival to adulthood to over 95% [4].

Newborn screening (NBS) began in the 1960s with Robert Guthrie's pioneering work, but universal screening for sickle cell disease was not widely introduced until the early 2000s in the United States, the United Kingdom, and later Brazil. Several European countries-such as the Netherlands, France, Portugal and Germany-have since adopted universal SCD screening, while others continue to operate pilot programs [1].

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Approximately twenty low and middle-income countries (LMICs) across sub-Saharan Africa (SSA), the Caribbean, South Asia, and the Middle East have reported newborn screening initiatives between 1973 and 2024, revealing a significant disease burden with sickle cell disease prevalence ranging from 0.8% to 1.9% [5,6], however the implementation is limited to institutional or regional programs.

Across SSA, since 1995, several countries, implemented SCD NBS, in settings with high SCD prevalence, ranging from urban tertiary care centers to rural primary health facilities. The programs operated across diverse contexts, few extended for decades [5], however, the majority were pilots, or at least regionals, but none were universal. Most programs were implemented in urban settings at tertiary or regional hospitals [7], though some successfully integrated screening into primary health-care centers [8] and reached rural communities [5].

The use of dried blood spots-the universal collection method-greatly facilitates specimen transport and storage, which is particularly important in many sub-Saharan African settings. Screening technologies have relied primarily on laboratory-based methods, with isoelectric focusing being the most used technique [5]. However, point-of-care testing has emerged as an important innovation, achieving in some studies 100% sensitivity and 100% specificity compared with HPLC [8].

In Angola, a West African country with a population of approximately 33.4 million, SCD is the most prevalent genetic disease and represents a significant public health concern [9]. It is estimated that around 21% of the population is a carrier of the sickle cell trait, resulting in an estimated prevalence of 1.5% for SCD [10].

This paper aims to describe the implementation of newborn screening for SCD at one of Luanda's largest maternity hospitals, the Hospital Materno Infantil Dr. Manuel Pedro Azancot de Menezes, together with the subsequent pediatric follow-up of diagnosed children. An additional objective is to determine the prevalence of haemoglobinopathies, including sickle cell trait carriers, within the Angolan population.

The Lancet Haematology Commission's recommendation about screening is that by 2025, policies, resources, and facilities are in place to allow all babies worldwide to be evaluated, meaning that Universal NBS should be a priority in SSA [1].

## 2. Materials and methods

### 2.1. Study design and participants

From June 2023 to December 2024, all children born at or vaccinated in Hospital Materno-Infantil Dr. Manuel Pedro Anzacot de Menezes, Angola, whose parents provided informed consent, were screened for Sickle Cell Disease. None of the parents refused to participate in the screening.

The study was approved by the Ethics Committee of the Ministry of Health of Angola and by the Ethics Committee of the Escola Superior de Saúde de Lisboa, Portugal (Parecer n° 21 CE/MINSA.INIS/2023 and CE-ESTeSL n° 28-2023). Written informed consent was obtained from the legal guardians of all newborns prior to participation.

Our objective from the outset was to include both children born in the hospital and those attending the vaccination room, which functions as a primary healthcare point. Because many births in the region take place at home, incorporating the vaccination room was essential to ensure broad coverage and ultimately increased the number of participants in the newborn screening programme.

### 2.2. Training of laboratory technicians

The implementation of newborn screening in Luanda was supported by the ARISE project, *African Research and Innovative Initiative for Sickle Cell Education: Improving Research Capacity for Service Improvement*, funded under the Horizon 2020 – Marie Skłodowska-Curie Actions – Research and Innovation Staff Exchange (RISE) 2018 Call.

Within this collaboration, the project facilitated the secondment of Angolan laboratory technicians to Lisbon (Portugal), where they were trained and carried out the laboratory procedures required for this programme, including isoelectric focusing (IEF) and genetic analyses relevant to newborn screening.

Training materials and standard operating procedures were developed for laboratory scientists and technicians. These resources were used to train staff in sample collection, screening methodologies, operation of the newborn screening bloodspot card system, puncher interface, and interpretation of results. In addition, hands-on training for the IEF equipment was provided by Revvity (Finland), the supplier of the system.

### 2.3. Sample collection, procedure, and storage

Blood samples were collected by heel-prick onto a purpose built collection card prepared with 3M filter paper, labeled with the date of collection and a number, and stored at −20 °C until processing. Using these locally made collection cards reduced costs and improved the sustainability of the procedure, as the technicians themselves produced the cards.

Haemoglobin electrophoresis was performed by isoelectric focusing (IEF) using the Migele™ IEF Platform (Revvity) acquired with the support of Revvity/PerkinElmer and the ARISE project-following the manufacturer's instructions. The laboratory analyses were carried out at the Human Genetics Laboratory of the Escola Superior de Saúde de Lisboa (ESSL), Lisbon, Portugal, and were performed mainly by laboratory technicians on secondment from Angola and Nigeria.

All samples identified as HbSS were genotyped for the HbS mutation by PCR–RFLP [11] following DNA extraction using the Chelex 100 resin method (Bio-Rad), according to the manufacturer's instructions. All atypical electrophoretic patterns suggestive of other haemoglobin variants were further analysed by Sanger sequencing.

Parents were informed of the IEF results during post-delivery hospital visits or at subsequent vaccination appointments. However, parents of infants suspected of having sickle cell disease were contacted directly by telephone. During this communication, they were advised of the need for a clinical consultation at the hospital and for a second blood sample to perform a confirmatory test by capillary electrophoresis (or genetic analysis, if required). Newly identified patients were then enrolled in a comprehensive care programme for sickle cell disease. All newborn screening tests and follow-up consultations were provided free of charge.

### 2.4. Statistical analysis

We calculated the proportion of newborns and infants with sickle cell anaemia (HbSS), carriers of the sickle cell trait (HbAS), carriers of HbC (HbAC), and those with normal haemoglobin (HbAA). The prevalence of any additional haemoglobin genotypes detected was also determined.

## 3. Results

This study included a total of 13,256 newborns who were either delivered at the hospital or attended the vaccination room within their first month of life between June 2023 and December 2024. The average weekly number of samples collected from births and vaccinations was approximately 160.

Bloodspot samples were collected from newborns and infants in two settings: 96% ($n = 12{,}662$) from the labour ward and 4% ($n = 594$) from the immunization room. The haemoglobin phenotype distribution identified by IEF showed HbFA in 78.22% ($n = 10{,}369$) and HbFAS in 20.31% ($n = 2692$), followed by the predominant SCD phenotype HbFS at 1.38% ($n = 183$). In 0.2% of samples ($n = 31$), additional haemoglobin variants were detected, including 8 HbFAC, 1 HbFAE (Table 1), and 19 with electrophoretic patterns on IEF compatible with α-globin

**Table 1**
Haemoglobin phenotypes observed in the screened infants. (X denotes electrophoretic abnormalities potentially related to the β-globin gene; however, no corresponding mutation was identified by sequencing.)

| Haemoglobin phenotype | N | (%) |
|---|---|---|
| AA | 10,369 | 78,22% |
| AS | 2692 | 20,31% |
| SS | 183 | 1,38% |
| AC | 8 | 0,06% |
| AE | 1 | 0,01% |
| AX | 3 | 0,02% |

gene alterations. The distribution of haemoglobin genotypes was consistent with Hardy–Weinberg equilibrium.

All 183 SS samples were analysed by PCR-RFLP to confirm the phenotype. In some cases, primarily in premature infants, the IEF pattern was inconclusive due to low protein quantity. For these 200 samples, DNA extraction and PCR-RFLP were performed. In 22 of those inconclusive IEF cases, the SS genotype was confirmed; in the others, AS or even AA patterns were obtained. In one case, a potential $\beta^0$-thalassemia was suspected, but no causative mutation was identified by Sanger sequencing. Additional atypical IEF patterns were also investigated by Sanger sequencing, which allowed confirmation of the C allele, the E allele, and other patterns associated with α-globin gene variants. We also detected the SNP rs72561473 in four samples. This SNP introduces an additional restriction site in the PCR-RFLP pattern, but it is considered a non-pathogenic variant.

A total of 106 (in 183) newborns with SCD (58%) identified through newborn screening at HMIPAM between June 2023 and December 2024 were successfully contacted. Some did not reply to phone calls (47, 26%), others the number given was not active (30, 16%) and just one declined further follow-up. Just 76 (42%) were enrolled in SCD follow-up initiated on free penicillin and antimalarial prophylaxis, as well as, provided with anticipatory guidance on splenic sequestration, fever management, and other preventive strategies. The hospital was not able to give free hydroxyurea to the patients. Several parents attended their first consultation only after the child experienced an initial pain crisis. We determined that two of the affected children had already died.

## 4. Discussion

Newborn screening is a public health system identifying conditions affecting a child's long-term health and survival. Specifically, in SCD, a condition that, if not treated shortly after birth, will result in severe lifelong disability, chronic complications or even death, NBS is of crucial importance. When followed by minimal care, which includes penicillin and vaccination, NBS for SCD reduces the occurrence of life-threatening complications and reduces under-five mortality [12].

Through the screening of 13,256 newborns over a 19-month period, we identified 183 cases of sickle cell disease, corresponding to a prevalence of 1.38% for HbSS and 20.31% for HbAS in Angola. These findings are consistent with previous data from Angola, namely the 1.51% prevalence reported by McGann et al [10]

Across sub-Saharan Africa, the prevalence of sickle cell disease varies markedly, with reported rates ranging from 0.2% to 2.8%. The lowest values have been observed in Beni and Butembo in the Democratic Republic of the Congo, with 0.2% HbSS [13], and 0.8% in Tanzania [14]. In contrast, one of the highest prevalences, 2.8% HbSS, has been documented in Nigeria [15]. Another study from the Democratic Republic of the Congo reported an HbSS prevalence of 1.4% [16]. In Mali, a sickle cell disease prevalence of 1.64% was reported, comprising 0.63% HbSS, 0.85% HbSC, and 0.16% HbS/$\beta^+$-thalassemia [17]. In contrast, no HbSC cases were detected in our Angolan cohort.

More recently, a large multi-country study conducted within the CONSA programme and involving seven sub-Saharan African nations (Ghana, Kenya, Liberia, Nigeria, Tanzania, Uganda and Zambia) reported a prevalence of 1.44% for SCD [18], a value remarkably similar to that found in the present study.

Isoelectric focusing (IEF) is the most used method in newborn screening for haemoglobinopathies; however, some centres use HPLC, and more recently, several programmes have successfully implemented point-of-care tests (POC) [17]. In a 2023 study conducted in Angola, 2000 newborns were screened using two POC tests, which demonstrated high accuracy (95.3%–98.3%) compared with isoelectric focusing. Immediate diagnosis and counseling at the point of care resulted in linkage to clinical care for 92% of infants [19].

Overall, IEF appears to be a more reliable, robust, and cost-effective method than capillary electrophoresis for SCD screening in sub-Saharan Africa [20]. IEF has standard commercial controls for accurate identification of haemoglobin variants, and is easy to observe and document. Long-standing screening programmes have demonstrated the effectiveness of IEF over several decades. In Jamaica, where IEF has been used since the 1970s, the programme achieved a 98% sample collection rate for SCD diagnosis [21]. However, several disadvantages of IEF can be identified. These include its labor-intensive nature, the need for appropriate laboratory infrastructure, dependence on consistent reagent supply chains, and the requirement for trained personnel [12].

Based on the 19 months of experience gained in Angola in the present study, IEF continues to demonstrate itself as a proven, cost-effective, and reliable technology for newborn screening of sickle cell disease in resource-limited settings, particularly when supported by adequate training, quality control, and standardized protocols.

However, POC testing offers several advantages over isoelectric focusing (IEF) for newborn screening of sickle cell disease in resource-limited settings. In Angola, POC tests demonstrated high diagnostic accuracy (95.3%–98.3%) compared with IEF, even in infants with high fetal haemoglobin, while enabling same-day diagnosis, counseling, and markedly improved linkage to care (92%) [19]. In contrast, centralized IEF testing is labor-intensive, requires specialized infrastructure, and is associated with delayed results and substantial loss to follow-up. Although POC tests cannot detect rare haemoglobin variants and may be subject to user-dependent errors, their real-world feasibility and clinical impact support their use as primary diagnostic tools for early sickle cell disease detection in high-burden, low-resource settings.

Staff training is a cornerstone of sustainable programme implementation. Within the ASH CONSA initiative, rigorous and recurring training and mentorship were provided both in person and virtually, including a hands-on refresher workshop in Ghana attended by two laboratory staff from each participating site [22]. In the present study, 27 laboratory technicians from Angola and Nigeria received training in IEF techniques and genetic analysis in Lisbon, delivered within the framework of the ARISE programme funded by the European Union. Similarly, Tanzania adopted a training-of-trainers model, complemented by regular refresher sessions for healthcare workers and laboratory personnel [14].

The greatest barrier to the long-term success and sustainability of NBS programmes resulted from their incomplete adoption into routine health systems [12]However, another important factor must be considered: dependence on external funding, which can threaten the long-term viability of newborn screening programmes. In our project, sustainability was jeopardized with the end of ARISE project funding and the hospital's limited capacity to independently support NBS activities. This challenge is largely driven by insufficient government commitment and public funding and challenges with programme ownership. Technical infrastructure challenges also pose threats to sustainability, particularly those related to equipment maintenance and the reliability of supply chains. In addition, human resource constraints remain a significant issue, including the shortage of skilled personnel and high staff turnover [14].

In our study, we successfully contacted 58% of the families of the identified Sickle Cell Disease cases, a proportion similar to that reported in a newborn screening program conducted in Luanda in 2013 (54.3%)

[23]. Moreover, follow-up compliance in that study was 96.6%, comparable to the rate observed in the present study (98,6%).Sustainability is also undermined by inadequate patient tracking and follow-up. For example, lost-to-follow-up rates in Ghana have ranged from 13.6% to 37.5% [5], largely driven by social and cultural barriers such as low disease literacy and stigma [24]. In our study, several parents declined follow-up consultations, often stating that their child had 'no symptoms'.

Taken together, the evidence shows that sustainable newborn screening programmes require several key elements: early government engagement with clear financial commitments; integration of screening into existing health systems rather than standalone initiatives; selection of technologies appropriate to local laboratory capacity; robust tracking systems with multimodal approaches to patient follow-up; continuous training and local capacity building; strong community engagement to improve disease literacy and address cultural barriers; and diversified funding strategies to reduce dependence on external support.

The majority of NBS programmes for SCD conduct sampling in maternity hospitals and birthing centres immediately after delivery [5]. However, several initiatives have successfully integrated screening into vaccination clinics, demonstrating substantial potential for expanding coverage. Notably, in Nigeria, screening was incorporated into routine primary healthcare immunization services across five centres, resulting in markedly improved reach [8].

In the present study, we also screened all infants attending their first vaccination visits at the hospital, enabling the inclusion of newborns delivered at home, since immunization programmes typically achieve higher population coverage than hospital-based delivery services. Similarly, the Tanzanian programme recommended that newborn screening be linked to immunization services to optimize resources and enhance feasibility, further supporting this integrated approach [14].

## 5. Conclusion

This study corroborates the estimates of high prevalence of SCD in Angola, 1.38% SS and 20.31%AS, thus demonstrating that the implementation of newborn screening for the early diagnosis of these patients is feasible and should be a priority.

Given the high childhood mortality associated with SCD, the World Health Organization highlights the disease as a priority under Sustainable Development Goal (SDG) 3: 'Ensure healthy lives and promote well-being for all at all ages.' Yet many sub-Saharan African countries face major resource and workforce constraints that limit newborn screening and timely care. Expanding access to newborn screening is therefore critical to advancing SDG targets on reducing maternal mortality, lowering newborn and under-five mortality, and decreasing premature deaths from noncommunicable diseases.

## CRediT authorship contribution statement

**Miguel Brito:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Catarina Ginete:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Mariana Jacinto:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Manuela Mendes:** Writing – review & editing, Methodology, Investigation. **Hailton Soares:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Roberto Afonso:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Adriano Siatembo:** Writing – review & editing, Methodology, Investigation. **Jocelyne Vasconcelos:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Baba Inusa:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization.

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## Declaration of competing interest

The authors declare that they have no competing interests.

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## Data availability

Deidentified data will be made available upon reasonable request to the corresponding author, contingent on a justified proposal and following institutional approvals.

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