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## EPIDEMIOLOGICAL EVALUATION OF THE NATIONAL SICKLE CELL SCREENING PROGRAM IN THE REPUBLIC OF UGANDA

ARIELLE G. HERNANDEZ

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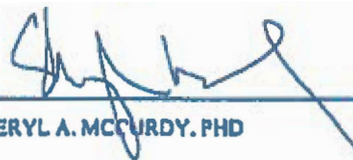
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Arielle G. Hernandez, MPH

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## DEDICATION

To my most near and dear ones: Mom, Dad, and Kali.

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REPUBLIC OF UGANDA

By

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in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

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EPIDEMIOLOGICAL EVALUATION OF THE NATIONAL SICKLE CELL SCREENING PROGRAM IN THE  
REPUBLIC OF UGANDA

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The University of Texas  
School of Public Health, 2019

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Sickle cell anemia contributes substantially to childhood morbidity and mortality in sub-Saharan Africa, where there are scarce health resources and inadequate awareness among healthcare providers and the community. Without early diagnosis and initiation of preventive treatments, most infants will die of acute complications before their fifth birthday. Information on the mortality associated with sickle cell anemia is excluded from global statistical summaries due to the lack of accurate data, making sickle cell anemia an invisible killer of children on the African continent.

Information from long-term large-scale sickle cell screening efforts is not yet available in Africa; therefore, a mixed-methods study was conducted using five years of data from Uganda's national sickle cell screening program and from interviews with 23 sickle cell healthcare providers. This was achieved with the following objectives: (i) characterize the epidemiology of sickle cell disease in Uganda; (ii) evaluate the centralized sickle cell screening laboratory in Uganda; and (iii) describe healthcare providers' experiences with sickle cell screening in Uganda.

A total of 324,356 children were screened for sickle cell trait and disease from February 2014 to March 2019. A high national burden of sickle cell disease (0.9%) was confirmed. Among samples referred specifically for sickle cell testing, the overall prevalence of sickle cell disease was 9.7% and particularly elevated in high-burden districts where focused screening occurred. A large proportion of affected children were tested between 5-9 months of age, coincident with onset of disease signs and symptoms. With the use of crude birth rate data, a high screening coverage of newborns was observed several high-burden districts. Median turnaround time from sample collection to result reporting was 16 days (IQR 11, 24). Predictors affecting prolonged turnaround time were sample volume, health facility level, and testing month. Cost per test was \$4.46 and cost per case detected was \$483.74. Barriers to screening were identified, including the need for initial and ongoing training for healthcare providers on sickle cell screening and management; healthcare system capacity issues, such as resources of staff and supplies, and system fragmentation; and the knowledge and awareness gap of sickle cell disease in the community.

Focused sickle cell screening has been a time- and cost-effective approach to begin to confront Uganda's large burden of disease. However, as the adverse impact of sickle cell disease on the population becomes more fully realized, a shift toward earlier and more widespread screening through healthcare provider training, community education, and improved sickle cell care models will be most advantageous. The experiences in Uganda are instructive for all countries in sub-Saharan Africa with a large sickle cell burden.

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## BACKGROUND

### *Molecular Basis and Pathophysiology of Sickle Cell Anemia*

Sickle cell disease refers to a group of genetic blood disorders that affects hemoglobin, the protein in erythrocytes responsible for transporting and delivering oxygen throughout the body. Sickle cell anemia, the most common and severe form of sickle cell disease, is a monogenic disorder caused by a homozygous mutation in the beta-globin gene on chromosome 11, due to a single nucleotide polymorphism (rs334) of adenine to thymine at the sixth codon position resulting in an amino acid substitution of glutamic acid for valine.<sup>1,2,3</sup> The beta-globin gene encodes the beta-globin protein, a critical subunit of the hemoglobin molecule.<sup>2</sup>

Normal adult hemoglobin (HbA) is a tetramer that consists of four protein subunits of two alpha-globin chains and two beta-globin chains. The mutation in the beta-globin chain that creates the abnormal variant sickle hemoglobin (HbS) causes the hemoglobin molecule to change conformation, due to the hydrophobic nature of valine compared to the hydrophilic glutamic acid (Figure 1). When deoxygenated, the HbS tetramer rapidly forms a polymer within the erythrocytes; this act of “sickling” in erythrocytes results in long, inflexible tactoids that irreversibly distort the cell membrane into the distinct crescent or “sickle” shape.

Polymerization of deoxygenated HbS is the primary molecular pathophysiologic event of sickle cell anemia, and downstream pleiotropic effects include alteration of the erythrocyte membrane structure and function, increased density, increased adhesion to vascular

endothelium, abnormal vasoactivity, and inflammation (Figure 2a and 2b).<sup>4,5</sup> These events contribute to sickle vaso-occlusion and hemolysis, which are considered to be the clinical hallmarks of sickle cell disease.

Additional sickle cell disease genotypes include heterozygosity between HbS and mutations that result in other structural variants of beta-globin (such as HbC) or reduced level of beta-globin production (beta-thalassemia). Individuals who inherit a single copy of the mutated HbS allele in combination with a normal HbA allele have the heterozygous HbAS genotype and do not have sickle cell disease; they are called carriers and have sickle cell trait, which has no phenotypic characteristics.

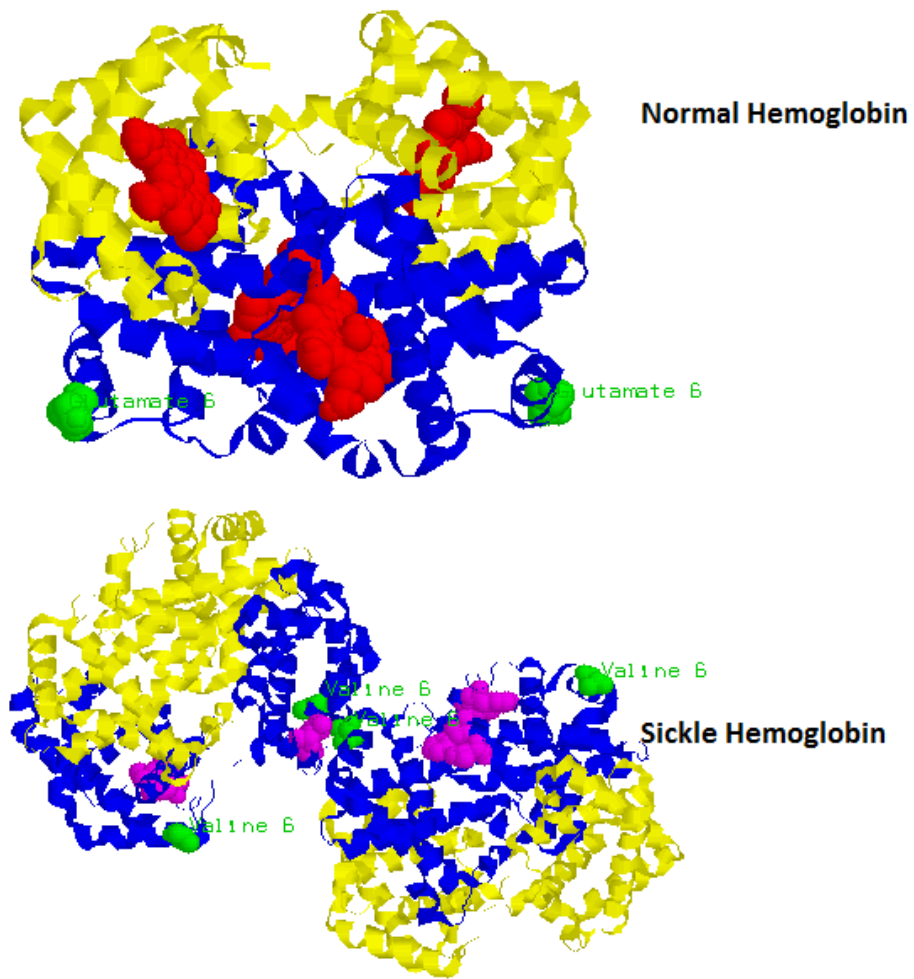


Figure 1: Hemoglobin in which glutamic acid is replaced with valine at position six.

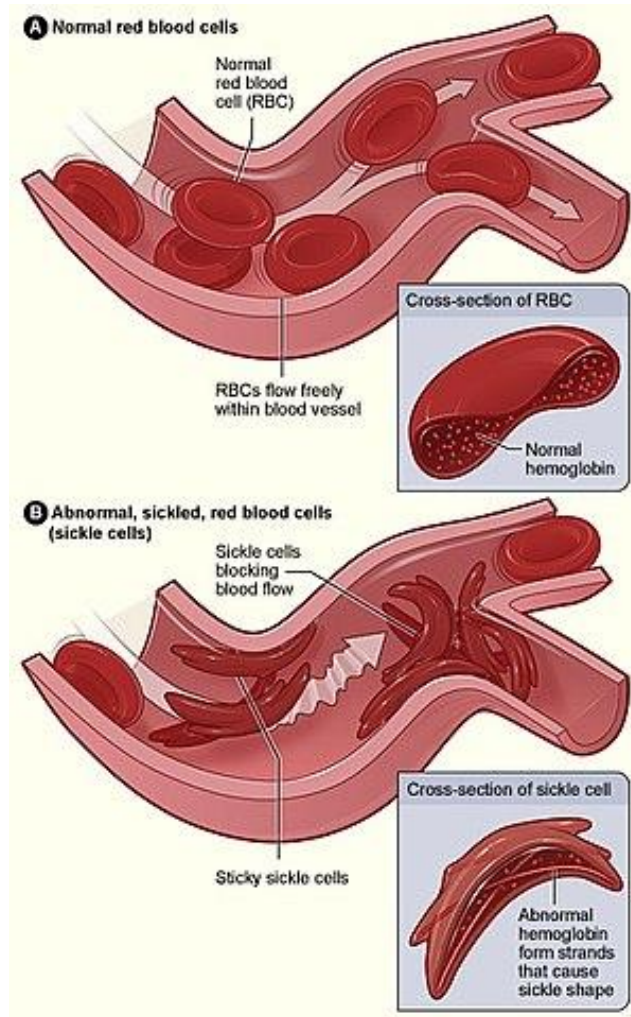


Figure 2: (a) Normal erythrocytes in circulation. (b) Sickled erythrocytes in circulation, portraying problems that arise from the sickle shape.

### *Origins of the Sickle Mutation*

Haplotype analysis suggests that the sickle gene arose, and independently amplified to its current population frequencies, on at least four occasions.<sup>6,7,8</sup> Distinct beta-globin haplotypes can be assigned to the HbS allele, based on flanking DNA sequences, which reflect the regions in sub-Saharan Africa, India, and the Arab peninsula where the mutation arose (Figure 3).

Despite being harmful in its homozygous form, the sickle allele has been observed to reach very high frequencies in areas that are now or have been endemic for malaria.<sup>9</sup> This observation led to the “malaria hypothesis”<sup>10</sup> that suggests the prevalence of sickle cell trait in high-pressure malaria regions reflects a survival advantage that the sickle gene offers (Figure 4a and 1b).<sup>9,11</sup> The sickle gene is thus, a classic example of a balanced genetic polymorphism, as it confers positive selection for heterozygous carriers (HbAS, sickle cell trait) who are relatively protected against *Plasmodium falciparum* malaria, but negative selection for homozygotes (HbSS, sickle cell anemia).<sup>12</sup> The increased resistance of individuals with sickle cell trait to *Plasmodium falciparum* malaria is considerable. In a meta-analysis of studies of severe malaria conducted throughout the African continent, it was estimated that children who were heterozygotes were more than 90% less likely to develop severe and complicated malaria compared to normal children with HbAA.<sup>13</sup> In a large multicenter case-control study of severe malaria across the malaria-endemic world, the odds ratio for severe malaria among children with sickle cell trait was 0.14 (0.12-0.16; p-value  $1.6 \times 10^{-225}$ ).<sup>14</sup>

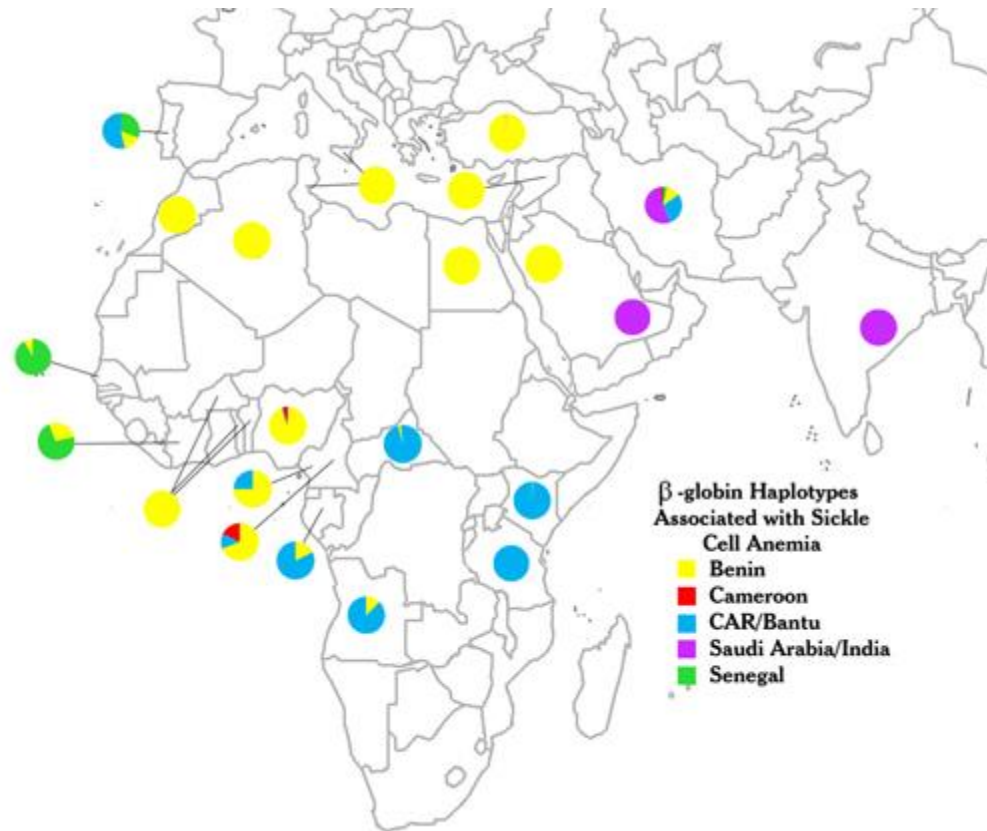


Figure 3: The distribution of sickle cell anemia haplotypes among countries with high prevalence of the disease. From Gabriel et al (129).

Various mechanisms underlying the protection of HbS against malaria have been described, including that the erythrocytes containing HbS impair parasite growth and multiplication when polymerized, and that parasitized HbS erythrocytes are more rapidly cleared from circulation through innate or acquired immune-mediated processes.<sup>15-21</sup> Studies have also suggested that HbS erythrocytes trigger reduced expression of the parasite-encoded protein *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) on the surface of malaria-infected erythrocytes disrupting endothelial adhesion.<sup>22,23</sup> Recently, it was reported that there is

selective expression in host HbS erythrocytes of two species of microRNA that integrate into the *Plasmodium falciparum* mRNAs to inhibit translation and thus, altering virulence.<sup>24,25,26</sup>

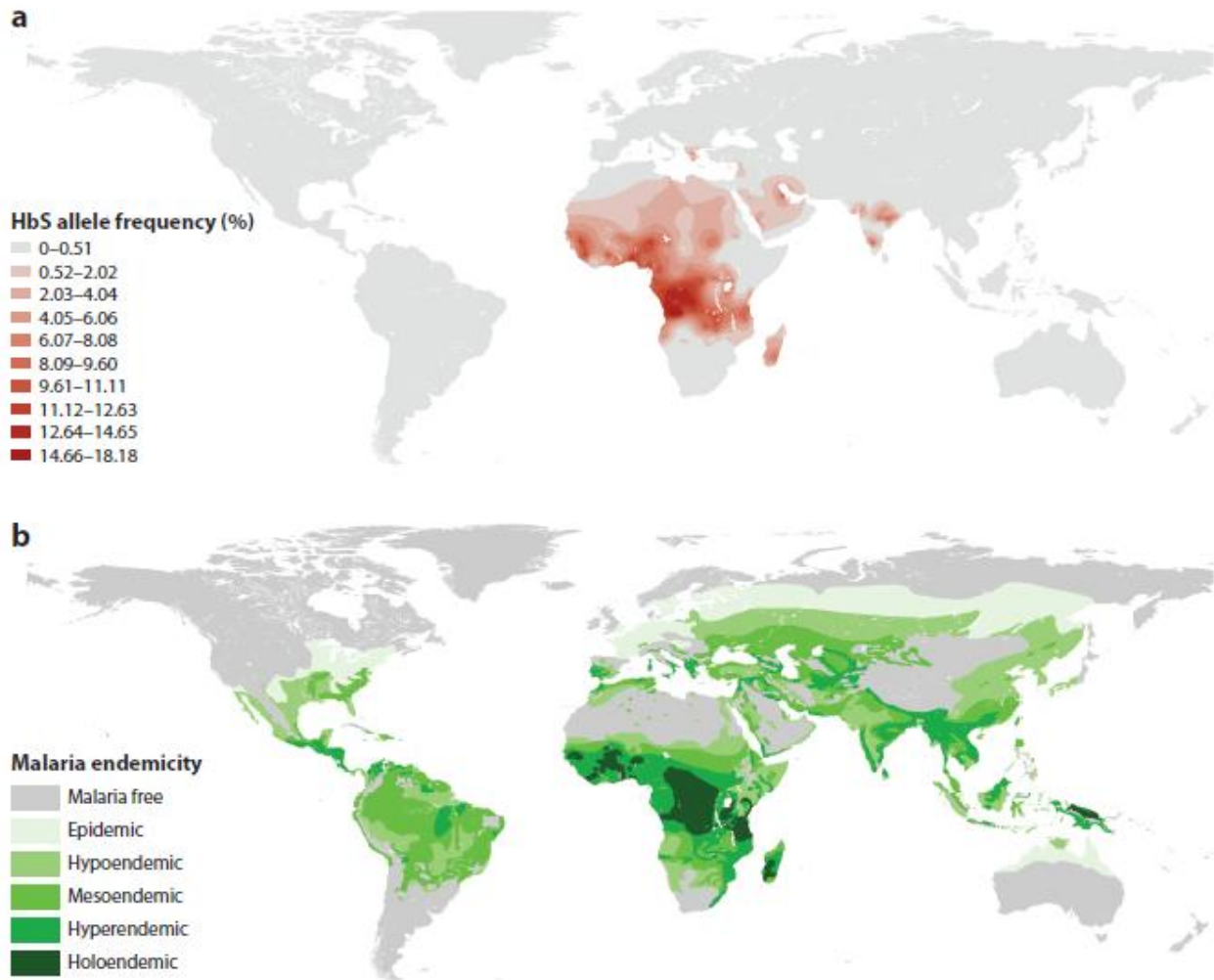


Figure 4: The global distribution of sickle cell anemia in relation to the historical distribution of *Plasmodium falciparum* malaria. (a) The geospatial distribution of the HbS allele. (b) Historical distribution of malaria. Figure adapted from Piel et al (8).

#### Global Burden and Distribution

Sickle cell anemia was the first recognized hemoglobinopathy and was described by James Herrick in 1910.<sup>27</sup> Later contributions by Linus Pauling in 1949 demonstrated that sickle cell disease was linked to an abnormality in the hemoglobin molecule by comparing different mobility of sickle hemoglobin compared to normal hemoglobin by gel electrophoresis.<sup>28,29</sup> Due to this discovery, sickle cell disease became known as the first “molecular disease” in the then emerging field of molecular medicine.<sup>30</sup> Subsequent studies unveiled the mode of autosomal recessive inheritance of the disease<sup>31</sup> and the discovery of the single amino acid substitution in the hemoglobin molecule of sickled cells.<sup>32</sup> By the mid-1960s, the genetics of thalassemias was generally known and several other hemoglobin variants had been characterized. In 1967, a fundamental compilation and interpretation of available data on hemoglobinopathies worldwide was published by Frank B. Livingston.<sup>33,34</sup> Fifteen years later, a World Health Organization (WHO) Working Group on the Community Control of Hereditary Anaemias of the Division of Non-Communicable Diseases released a report presenting diagnostic summaries, clinical features, and treatments for the most important hemoglobinopathies, emphasizing thalassemias and sickle cell anemia.<sup>35,36</sup> This was followed by WHO guidelines in 1994 and epidemiologic summaries by Modell, Angastiniotis, and others detailing the health burden of these diseases and measures for controlling them.<sup>37,38,39,40</sup> In 2008, Modell et al conducted a global epidemiological study of hemoglobin disorders and found that 5.2% of people were carriers of a significant hemoglobin variant. Africa was most severely affected, with almost 18.2% of the population carrying a significant hemoglobin variant and approximately 1% of all births were affected by sickle cell disorders, including HbSS, HbSC, and HbS/beta-thalassemia.

Furthermore, hemoglobinopathies contributed to 6.4% of the under-five mortality in sub-Saharan Africa.<sup>41</sup>

In 2013, Piel et al published a map of HbS allele frequency and a summary of the epidemiology of HbS among newborns globally by creating a database of spatially located HbS surveys supplemented with data from the Malaria Genomic Epidemiology Network Consortium.<sup>11</sup> They estimated that 75.5% the annual 312,302 global sickle cell disease births occur in sub-Saharan Africa. At the country level, 30% of the total sickle cell trait and sickle cell disease in neonates were born in two sub-Saharan African countries, Nigeria and the Democratic Republic of Congo (Table 1). By 2050, it is projected that there will be a 30% increase in the number of sickle cell disease births globally, as well as an increase to 88% in the proportion of sickle cell disease births occurring in sub-Saharan Africa (Figure 5).<sup>12</sup>

Table 1: Summary of global and regional annual predicted estimates of HbAS and HbSS in neonates. Adapted from Piel et al (11).

	<b>HbAS in neonates/year Median (IQR)</b>	<b>Percent of Burden</b>	<b>HbSS in neonates/year Median (IQR)</b>	<b>Percent of Burden</b>
<b>Global</b>	5,476,407 (5,290,779-5,679,288)		312,302 (294,307-329,729)	
<b>Americas</b>	386,430 (349,253-425,791)	7.4	13,309 (10,869-15,210)	4.6
<b>Arab-India</b>	1,147,477 (1,010,443,1,299,147)	22.7	46,826 (39,147-56,000)	16.9
<b>Eurasia</b>	256,163 (216,499-310,758)	5.4	7,493 (5919-10,090)	3.0
<b>Southeast Asia</b>	2,535 (1,324-5,171)	0.1	21 (7-63)	0.0

<b>Sub-Saharan Africa</b>	3,580,207 (3,473,117-3,684,718)	64.4	235,681 (220,993-250,568)	75.5
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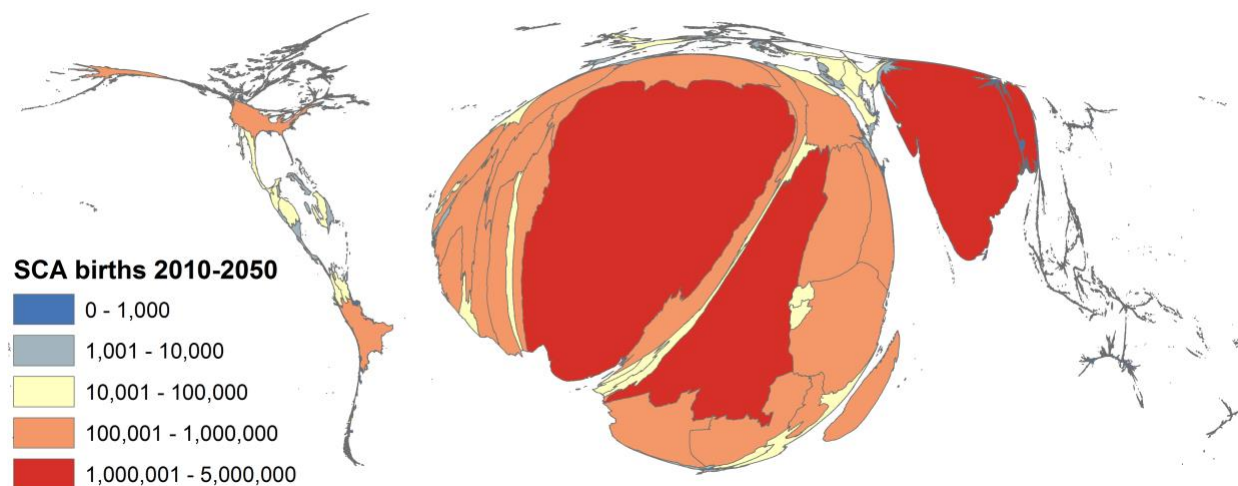


Figure 5: Global distribution of HbSS with the size of countries scaled to the number of annual births. From Piel et al (12).

### *Diagnosis*

Interventions such as newborn screening, which allows early diagnosis followed by initiation of care and treatment, have had significant impact on improving the quality of life and survival for individuals with sickle cell disease in high-income countries.<sup>42</sup> In the United States and some European countries, newborn screening identifies affected but still asymptomatic babies, allowing for early initiation of important treatments such as penicillin prophylaxis and pneumococcal immunizations before severe, life-threatening disease complications arise.<sup>43</sup> These same interventions can be achieved in Africa, where national newborn screening does not currently exist, but where programs and improvements to basic healthcare will benefit the

greatest number of individuals with sickle cell disease.<sup>44</sup> WHO has recommended newborn screening as a key strategy for reducing pediatric mortality in Africa,<sup>45</sup> where infants with sickle cell disease face an estimated 50-90% early childhood mortality rate.<sup>46</sup> Newborn sickle cell screening is relatively new in Africa with pilot studies in a few countries including Angola,<sup>47</sup> Benin,<sup>48</sup> Ghana,<sup>49</sup> Democratic Republic of Congo,<sup>50</sup> Burkina Faso,<sup>51</sup> Tanzania,<sup>52</sup> Liberia,<sup>53</sup> and Nigeria.<sup>54</sup> Data from these local experiences have mounted evidence of the importance and feasibility of sickle cell newborn screening in Africa, however, information from long-term screening efforts is not yet available.

The most popular methods for diagnosing sickle cell disease are isoelectric focusing (IEF), capillary zone electrophoresis (CZE), and high-performance liquid chromatography (HPLC). Isoelectric focusing is a robust method adopted for newborn screening which allows the simultaneous testing of about 80 samples per plate. This procedure uses an electrical current to allow different hemoglobins to migrate and then “focus” to each unique isoelectric point on an agarose gel. By examining the predictable migration patterns at just a glance, HbS and other hemoglobin variants can be easily distinguishable from HbA and HbF to establish diagnosis. While IEF, CE, and HPLC are all sensitive in identifying individuals who have disease, they cannot reliably differentiate HbSS from a compound heterozygous, such as HbS/beta-thalassaemia. More advanced techniques, such as tandem mass spectrometry,<sup>55</sup> DNA diagnostics, and next-generation sequencing analysis,<sup>56,57</sup> have been adopted to address these limitations. These methods are employed in high-income countries for newborn screening, clinical practice, and research, but unavailable in low- and middle-income countries. A simple sickle solubility test

that uses sodium metabisulfite has been used for many years in these counties, but cannot accurately distinguish sickle cell trait from disease. Increasingly sophisticated point-of-care devices hold promise for widespread testing in low-resource settings, such as the sickle cell disease immunoassay Sickle SCAN.<sup>58,59,60</sup>

### *Complications and Co-Morbidities*

Sickle cell disease has a wide range of complications that can affect every organ system in the body. Sickle cell disease presents in infancy with repeated infections and pain due to vaso-occlusive crisis, an obstruction of blood vessel circulation by sickled cells. In infants, an early sign of the disease is often swelling of the hands and feet, known as hand-and-foot syndrome or dactylitis. The many forms of vaso-occlusive crisis, such as acute chest syndrome, stroke, acute renal failure, priapism, and splenic sequestration, are very painful and can become life-threatening at any age. Sickle cell disease patients also experience chronic hemolytic anemia, caused by the rapid breakdown of the unstable HbS erythrocytes, resulting in regular fatigue and jaundice. Severe hemolytic anemia can become very dangerous for patients, especially in malaria endemic regions, and long-term outcomes include gallstones and pulmonary hypertension. Other chronic co-morbidities such as avascular necrosis, leg ulcers, diastolic heart dysfunction, and end-stage renal disease occur with increasing age.<sup>61,62</sup>

Bacterial infections are a major cause of morbidity and mortality in children with sickle cell disease. Recurring vaso-occlusive infarcts within the spleen impair the organ's function,

predisposing disease patients to severe infections with malaria and encapsulated bacteria like *Haemophilus influenzae*, *Streptococcus pneumoniae*, and non-Typhi Salmonella species.<sup>63,64,65</sup> Other immunological abnormalities, such as reduced serum opsonin activity and defects in complement activation, further increase susceptibility to other common organisms including *Mycoplasma pneumoniae*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Escherichia coli*.<sup>63</sup> Tissue ischemia and inflammation, and chronic transfusion also contribute to susceptibility.<sup>63</sup> In high-income countries, it is well documented that the introduction of penicillin prophylaxis and immunization with conjugate vaccines against *S. pneumoniae* and *H. influenzae* type b have led to substantial improvements in the prognosis of patients born with sickle cell disease.<sup>66-69</sup> In Africa, available data suggest different causative organisms threatening children with sickle cell diseases, such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, and non-Typhi Salmonella infections, but a lack of both *Streptococcus pneumoniae* and *Haemophilus influenza*.<sup>70-74</sup> Many of these studies are potentially biased due to diagnostic limitations and a focus on patients with existing sickle cell disease resulting in underreporting. These observations introduced wary criticism of the potential benefits of antibiotic prophylaxis in sickle cell disease patients in Africa. However, a case-control study in Kenya revealed that young children with sickle cell disease had high incidence of bacteremia from three common organisms: *Streptococcus pneumoniae*, *Haemophilus influenza*, and non-typhi *Salmonella* species. The incidence of pneumococcal bacteremia of 1.2-5.0 episodes per 100 person-years was similar to that reported from high-income countries (1.5-11.6) prior to introduction of antibiotic prophylaxis or pneumococcal vaccines.<sup>75</sup> Overall, the limited and contradictory data across Africa are problematic when it comes to streamlining and translating

evidence-based prevention strategies. Therefore, comprehensive studies designed to identify co-infecting pathogens of greatest threat to children with sickle cell disease in these countries is needed to inform guidelines and policy. Our limited knowledge of the pathogenesis of infections in sickle cell disease patients is a further hindrance. Recent studies have discussed increased gut permeability and microbiota alterations in sickle cell disease patients as a result of hypoxemia induced by recurrent sickling in the splanchnic vasculature.<sup>76-80</sup> The gut may therefore be another important susceptibility factor, as a potential portal of entry for pathogens into the systemic circulation and may mediate certain disease outcomes.<sup>80</sup> This prompts investigation into microbiota-based therapeutic approaches to prevent certain infections in sickle cell disease patients.

In Africa, it is necessary to consider the geographic overlap of sickle cell disease and HIV and the potential of an important syndemic. It is suspected that the sickle cell disease and HIV co-morbidity has an additive effect on mortality due to risk of overwhelming bacterial infections if untreated, but more complex pathophysiological interactions have been discussed. For example, an increased HIV burden may exist in circulating T-lymphocytes due to the lack of splenic lymphoid tissue following sickle-related organ infarction early in life. Alternatively, systemic inflammation that occurs during HIV infection may promote vaso-occlusion, leading to more severe sickle-related complications.<sup>81</sup> Conversely, sickle cell disease may uniquely impact HIV disease course by several mechanisms, such as enhanced immunity that may inhibit HIV viral replication due to persistent upregulation of inflammation, iron metabolism and immunologic changes in sickle cell disease.<sup>81</sup> Overall, these differing perspectives suggest that

further investigation of the roles sickle cell disease and HIV play is needed to help in health planning and policy across Africa.

### *Genetic Modifiers*

Genetic factors are responsible for the notoriously variable clinical course of sickle cell disease. Increased levels of fetal hemoglobin (HbF), and genetic loci associated with this trait, have been shown to influence the sickle cell disease phenotype.<sup>82-89</sup> HbF is composed of two alpha-globin chains and two gamma-globin chains and is the principal hemoglobin produced during fetal development. After birth, the gamma-chains are replaced by beta-chains and the switch to adult hemoglobin occurs, making HbA predominate with trace amounts of residual HbF. In sickle cell disease, HbF persists and has the ability to hinder deoxygenated HbS polymerization, which has been shown to be protective against several disease complications at high concentrations.<sup>82</sup> Of the five common beta globin sickle haplotypes, patients with the Bantu haplotype have the lowest HbF, while those with the Senegal or Arab-Indian haplotypes have the highest HbF, which is associated with milder but not asymptomatic disease.<sup>82,83</sup> HbF levels differ among and within all haplotypes, signifying the importance of other quantitative trait loci for HbF heterogeneity. Genome-wide association studies have unveiled the presence of HbF inducing genotypes at the three major quantitative trait loci for HbF persistence and these include *BCL11A* on chromosome 2, *HMIP-2* on chromosome 6, and *Xmn1-HBG2* on chromosome 11.<sup>90,91</sup> At each locus, strong candidates for a causative functional DNA change

have been proposed and are potential targets of therapeutic strategies for HbF induction in sickle cell disease.

Co-inheritance of sickle cell disease and alpha-thalassemia, most commonly due to the deletion of either one or two alpha globin genes, is associated with reduced sickle hemoglobin polymerization and consequent hemolytic anemia due to decreased intracellular hemoglobin concentrations.<sup>92</sup> Alpha-thalassemia has been associated with a milder phenotype in sickle cell disease patients, but could also lead to increased vaso-occlusive pain<sup>93</sup> and avascular necrosis.<sup>94</sup> Another known genetic modifier of sickle cell disease is Glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-linked disorder that is highly polymorphic and among the most common genetic disorders identified in humans.<sup>95</sup> In affected individuals, the defect in the G6PD enzyme causes premature breakdown of erythrocytes and can cause severe jaundice in newborns. G6PD co-occurring with sickle cell disease is likely frequent and problematic as they both cause hemolysis. In Africa, high prevalence of alpha-thalassemia and G6PD deficiency have been reported,<sup>96,97,98</sup> and attributed to their protective effect against malaria. Further insight to document the frequency, pattern of co-inheritance with sickle cell disease, and bearing on clinical severity among patients by these two disorders in Africa is needed. Overall, HbF concentrations and co-inheritance of alpha thalassemia and G6PD deficiency are still not sufficient to explain the clinical diversity of sickle cell disease. Therefore, both candidate gene and genome-wide association studies have been used to search for other genetic associations with specific sickle cell disease complications, such as acute pain, bilirubin levels, and

predisposition to gallstones, stroke, priapism, avascular necrosis, leg ulcers, pulmonary hypertension and acute chest syndrome, bacteremia and other infections, and renal disease.<sup>99</sup>

### *Treatment*

Over the past 40 years, many prospective studies have collectively documented the effectiveness of penicillin prophylaxis and pneumococcal vaccination to prevent early death, as well as the benefits of acute and chronic transfusions and hydroxyurea therapy as established treatments for sickle cell disease in high-income countries.<sup>66,67,68</sup> In Africa, beyond the standard treatments that will continue to be needed for the prevention of malaria and bacterial infections, the increasing prevalence of sickle cell disease in these countries will progressively burden healthcare systems and communities without the wide-spread availability of disease-modifying therapies. Blood transfusions therapy is used to alleviate anemia and acute vaso-occlusive complications, and is a primary prevention of stroke in patients with abnormal transcranial Doppler velocities.<sup>100-105</sup> Despite these benefits, blood transfusion therapy does come with the risks of iron overload, transfusion-acquired infections, and risk of alloimmunization.<sup>104,105</sup> It is additionally quite costly and time consuming for patients.

Another disease modifying therapy for sickle cell disease is hydroxyurea, a once-daily oral medication to induce fetal hemoglobin production and help improve the laboratory and clinical complications of sickle cell disease. In the United States, hydroxyurea is the recommended treatment for patients with sickle cell disease and is listed on the WHO's Model List of Essential

Medicines for Children<sup>106</sup> and adults,<sup>107</sup> but is not yet widely available in Africa due to lack of local clinical evidence and experience with its use for sickle cell disease. To address this, there are several ongoing clinical trials that are generating critical data about the safety, efficacy, feasibility, and benefit of hydroxyurea for children with sickle cell disease in this setting. The REACH study (Realizing Effectiveness Across Continents with Hydroxyurea) is a prospective open-label dose escalation clinical trial that is currently treating about 600 children total in four African countries, including the Democratic Republic of Congo, Kenya, Angola, and Uganda. After three years of treatment, participants experienced improvements in several laboratory variables, including significant increases in hemoglobin and fetal hemoglobin. Compared with baseline, the rates of clinical adverse events were reduced from 308.4 to 170.7 events per 100 patient-years, including vaso-occlusive pain, malaria and non-malaria infections, transfusion, and death.<sup>108,109,110</sup> NOHARM (Novel use Of Hydroxyurea in an African Region with Malaria) examined the malaria incidence and severity in children with sickle cell anemia treated with hydroxyurea versus placebo in approximately 200 randomized children at the Mulago Hospital Sickle Cell Clinic in Kampala, Uganda. Compared with controls, the use of hydroxyurea was not associated with an increased incidence of malaria or other serious adverse events. Patients on hydroxyurea therapy had a lower rate of a composite clinical endpoint that included vaso-occlusive pain, dactylitis, acute chest syndrome, splenic sequestration, or transfusion (p-value = 0.001).<sup>111,112</sup> An extension of this study is underway to determine maximum tolerated dose and monitoring of hydroxyurea treatment for patients in this setting. These studies provide the necessary evidence that children with sickle cell disease across Africa will benefit from

hydroxyurea to prevent morbidity associated with the disease, however, the implications are limited by the costs of the treatment and laboratory monitoring that is needed.

The only curative sickle cell disease treatment is allogeneic hemopoietic stem-cell transplantation (HSCT).<sup>113</sup> The decision to pursue HSCT is complicated due to the risks and benefits tradeoff. At this time, HSCT is not a viable option in Africa due to limited infrastructure to support the procedure and follow-up, as well as the high cost for patients. Blood transfusion therapy and hydroxyurea are the most appropriate, potentially available, and likely to succeed in these countries.

## **Preliminary Data**

### *Sickle Cell Disease in Uganda*

Seventy years ago, a study by Lehmann and Raper reported the prevalence of sickle cell trait among different tribes compared with their physical anthropology in Uganda. They found the differences to be statistically significant, ranging from low prevalence (<5%) among Hamitic tribes, to more than 20% for many of the Nilotic tribes in the north, and a wide range among Bantu tribes with Bamba being the highest at 45% in the west.<sup>114</sup> A more recent but limited study of Uganda in 2010 suggested estimates much lower, reporting a prevalence of 3% to 17% for sickle cell trait and 0% to 3% for sickle cell disease in five districts in Eastern and Western Uganda.<sup>115</sup> While both studies attempt to quantify the burden of sickle cell disease in Uganda,

neither provides widespread geographic coverage of the country or adequate sample sizes for generalizability.

In 2006 and again in 2010, WHO issued a report highlighting the serious public health implications of sickle cell disease in Africa, acknowledging the inadequate national sickle cell policies and programs.<sup>46,116</sup> The WHO sickle cell strategy for the African region outlines several priority interventions focused on identifying the scope of the sickle cell disease burden within these countries, including strengthening of laboratory and diagnostic capacity and supplies with nationwide coverage; initiating and enhancing surveillance with monitoring and evaluation at the national level, and dissemination of results for policy-making; early identification and screening, ideally as part of routine newborn screening; and research promotion. It is further proposed that these interventions be guided by the principles of country ownership, leadership, and cultural sensitivity; integrated evidence-based and prevention approaches that are cost-effective and accessible and are partnership and team building in nature.

In response to the WHO guidelines, the Uganda Ministry of Health, Makerere University College of Health Sciences, and Cincinnati Children's Hospital developed a partnership to design and execute a national surveillance study of sickle cell disease in Uganda. The Uganda Sickle Surveillance Study (US3) was conducted to determine the prevalence and distribution of sickle cell trait and sickle cell disease in Uganda. To support this objective, the short-term goals of US3 were to build local sickle cell laboratory capacity within the Ministry of Health's existing

centralized laboratory infrastructure and to determine the feasibility of testing a high-volume of samples for large-scale long-term screening. At the Central Public Health Laboratories (CPHL) in Kampala, Uganda, a sickle cell laboratory was constructed and outfitted with isoelectric focusing equipment, and local personnel were recruited and trained on a technical isoelectric focusing protocol adapted for the study and newborn screening. A process of integrated testing and result reporting was established with the Centers for Disease Control sponsored Early Infant HIV Detection (EID) program at the CPHL, a program for prevention of mother-to-child transmission of HIV that identifies and tests approximately 100,000 HIV-exposed infants each year. As part of the EID program, blood samples are routinely collected by heel stick or finger stick on a standard dried blood spot card from newborns at health facilities and from children under two years at health facilities during immunization visits and/or during inpatient or outpatient visits. The samples are transferred to the CPHL via the national sample transport system for HIV testing<sup>117</sup> by viral polymerase chain reaction and for sickle cell disease testing by isoelectric focusing hemoglobin electrophoresis.

In the one-year cross-sectional US3 study,<sup>118</sup> isoelectric focusing was performed on a total of 99,243 consecutive dried blood spots collected in the EID program from HIV-exposed children in the sickle cell laboratory at the CPHL to identify the presence of sickle cell trait, sickle cell disease, and other hemoglobin variants. US3 documented a national prevalence of sickle cell trait of 13.3%, ranging from 4.6% in the South Western region to 19.8% in the East Central region. The overall sickle cell disease prevalence was 0.7%, with a range of 0.2% to 1.5% in the South Western and East Central regions, respectively. All districts in Uganda had sickle cell trait.

Eight districts, all within the South Western region, had <5% prevalence with the smallest proportion in the Mitooma district at 2.5%. There were eight districts with sickle cell trait prevalence >20% located in the East Central, Mid-Western, and Mid-Northern regions. The highest prevalence rate was at 23.9% in Alebtong district in the Mid-Northern region. Figure 6 is a district map of Uganda that illustrates the prevalence of sickle cell trait in 112 districts.

To investigate the hypothesis that areas of high sickle gene frequencies are coincident with currently and historically high levels of malaria due to the process of natural selection for the sickle gene, a statistical comparison of the regional prevalence of sickle cell trait and malaria was done. Malaria prevalence was based on the Uganda Bureau of Statistics 2009 malaria indicator survey data (the percentage of children 0-59 months of age with positive microscopy for malaria), ranging from 4.9% in Kampala and 11.6% in the South-Western regions to 62.5% in the Mid-Northern region.<sup>15</sup> The linear regression of numerical data by region resulted in a strong positive correlation between sickle cell trait and malaria in Uganda ( $r^2=0.69$ ,  $p=0.026$ ).

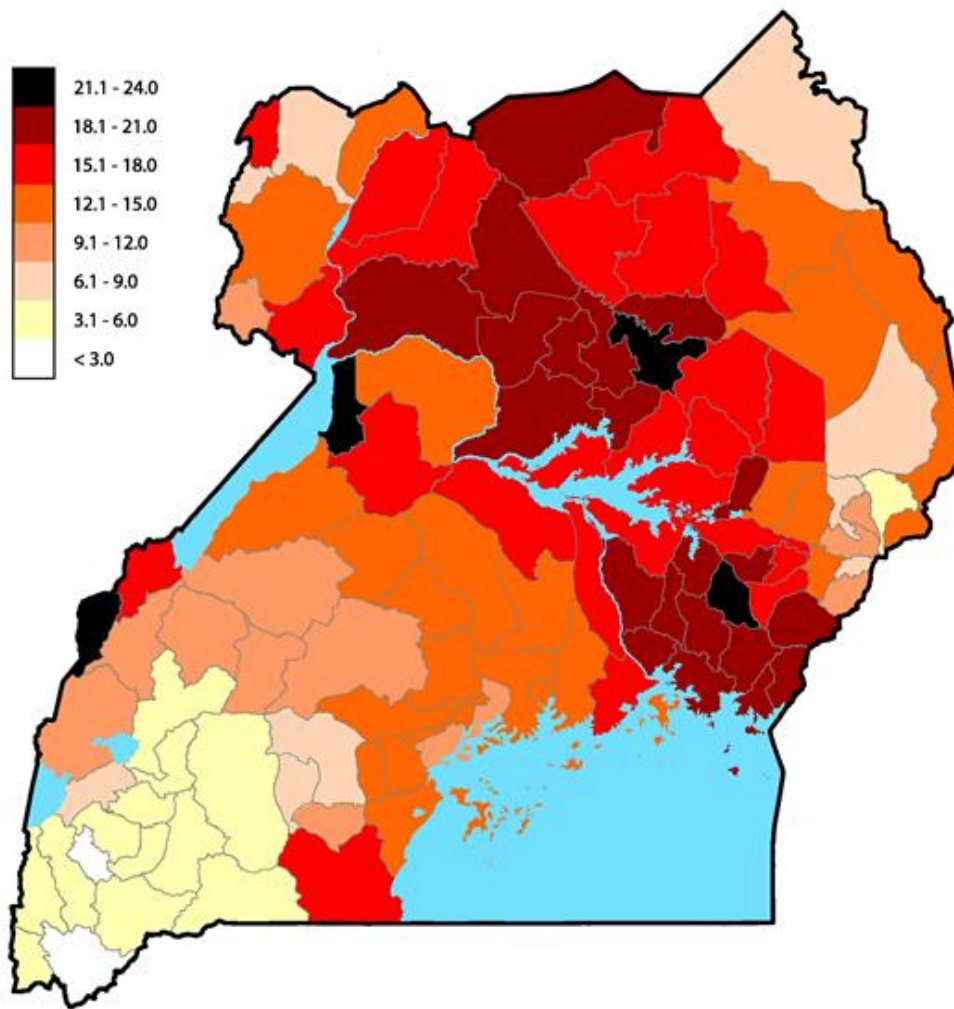


Figure 6: District map of the prevalence of sickle cell trait in Uganda. From Ndeezi et al (118).

The association between sickle cell disease and early mortality in children in sub-Saharan Africa is frequently cited.<sup>12,43,45,119-121</sup> It is estimated that the contribution of hemoglobin disorders to under-five deaths is 3.4% worldwide and 6.4% in Africa.<sup>38</sup> In countries like Uganda that experience higher HbS allele frequencies and lower overall childhood mortality, sickle cell disease may contribute up to 15% of the country's under-five mortality rate.<sup>43</sup> In the US3 study, a majority of the samples were from children below 18 months of age (99.3%). The data

revealed a decrease in the prevalence of sickle cell disease with increasing age (Table 2).

Specifically, the odds ratio at age 12.1-18.0 months compared to age 0.0-6.0 months was 0.79 (95% CI 0.64-0.97), which is consistent with early mortality for babies born with sickle cell disease in Uganda.

The prevalence of sickle cell disease compared to HIV status (Table 2) identified co-morbidity with early mortality for HIV in babies with sickle cell disease. The odds ratio of having sickle cell disease among HIV-positive infants, compared to HIV-negative infants, was 0.60 (95% CI 0.40-0.91). The low odds ratio highlights an important potential co-morbidity between sickle cell disease and HIV that results in early death. As discussed, this observation is not fully supported by the current published medical literature, where the interaction between sickle cell disease and HIV has been described.<sup>81</sup> This gap in knowledge is critical and our observations warrant prospective investigation of the overlap of two important diseases that heavily impact the same geographical areas.

Table 2: Sickle cell trait and sickle cell disease prevalence by age and HIV status in Uganda. From Ndeezi et al (118).

	TRAIT (%)	DISEASE (%)	TOTAL
<b>Age (m)</b>			
<b>0.0 – 6.0</b>	8802 (13.20)	521 (0.78)	66670
<b>6.1 – 12.0</b>	1758 (13.34)	87 (0.66)	13182
<b>12.1 – 18.0</b>	2326 (13.62)	105 (0.61)	17075

HIV Status			
<b>Negative</b>	12293 (13.36)	693 (0.75)	92024
<b>Positive</b>	672 (13.23)	23 (0.45)	5080

The Uganda Sickle Surveillance Study (US3) was the first national-level surveillance study of sickle cell disease to be conducted in Africa. The study documented a high and largely disproportionate prevalence of sickle cell trait and disease in Uganda. Analysis by region revealed that the observed sickle cell trait prevalence positively correlated with published malaria prevalence. Sickle cell disease was less common in children older than 12 months and those who were HIV-positive, consistent with early mortality and co-morbidity. The major strengths of the study were the (i) efficient and cost-effective design that was embedded within the existing infrastructure of the EID program; (ii) national sample transport system; (iii) a nationally representative cohort and large sample size for detailed analysis at both the district and regional levels; (iv) establishing a local sickle cell laboratory capacity; and (v) a multi-disciplinary partnership between government and academia. The main limitations were that the design did not allow collection of longitudinal data and the inability to ensure that all infants identified with sickle cell disease in the study received optimal clinical care due to the current lack of sickle cell care guidelines and knowledge among healthcare providers. Although further studies are needed to assess the current and future healthcare needs of children with sickle cell disease, the epidemiological data derived from this study directly inform national public health planning and policy to address the sickle cell burden within Uganda and other sub-Saharan African countries.

### *Sickle Cell Screening in High-Burden Districts*

Immediately following US3, the Uganda Ministry of Health launched a sickle cell screening program in identified high-burden districts. Screening commenced in two of the highest burden and largely populated districts in the Mid-Northern region, Gulu with a sickle cell trait prevalence of 19.9% and disease of 1.0% and Lira with a sickle cell trait prevalence of 19.8% and disease of 1.6%, and then expanded to include 18 districts by late 2017. To confirm the high sickle cell burden across the country, a total of 163,334 dried blood samples were analyzed at the sickle cell laboratory between April 2015 and March 2018, including 112,352 samples collected within the EID program and 50,982 additional samples collected specifically for sickle cell testing.<sup>123</sup> The prevalence of sickle cell trait and disease within the HIV-exposed EID cohort age 24 months or younger was >18% and >1%, respectively, which was analogous to the original US3 data (Table 3). Children six months or younger have a sickle cell trait prevalence of 14.4% and a sickle cell disease prevalence of 1%. With this information and based on the crude birth rate data from the Uganda Bureau of Statistics for each district, an estimated 236,905 sickle cell trait births and 16,695 sickle cell disease births occurred in Uganda in 2018. A follow-up investigation of the sickle cell disease and HIV co-morbidity and its association with increased mortality found that the prevalence of HIV-positive children with sickle cell disease was again significantly less than in HIV-negative children, with an odds ratio of 0.50 (95%CI=0.40-0.64).

Estimation of the frequency of known genetic modifiers of sickle cell disease was conducted, specifically alpha-thalassemia trait, G6PD deficiency, and beta-globin haplotype. 264 dried blood spots with documented sickle cell disease from the CPHL sickle cell laboratory were randomly selected, collected from either the original US3 or from the newborn screening program. All of these samples were first confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) to be homozygous HbSS, followed by genotyping for alpha-thalassemia trait, G6PD deficiency, and beta-globin haplotype. The prevalence of alpha-thalassemia trait was very high at 46% (40% with one-gene deletion and 6% with two-gene deletion). The most common G6PD variant in individuals of African descent is the A<sup>-</sup> variant, which was identified in 14% of males (all hemizygotes) and 32% of females (30% heterozygotes and 2% homozygotes). The distribution of alpha-thalassemia trait and G6PD deficiency differed from each other and from the sickle trait distribution. This suggests negative epistasis, in which the combined effect of beneficial mutations is smaller than would be expected from their separate effects. Beta-globin haplotype determination revealed that almost all of the samples were homozygous Central African Republic (CAR, Bantu), considered the most severe haplotype, with a few of the Arab haplotype found.

The epidemiological data derived from US3 and the subsequent three-year post-US3 newborn screening program has firmly established that the burden of sickle cell disease in Uganda is high and represents an impactful syndemic in combination with the HIV burden. Furthermore, documenting frequency and distribution of known genetic modifiers of sickle cell disease is prognostically important to guide clinical care. Sickle cell newborn screening is the ideal high-

impact and cost-effective strategy for high-burden low-resource settings. Early identification will progress affected children into long-term follow-up care and, over time, will help determine incidence and monitor survival for the development and improvement of clinical programs and policy for sickle cell disease in sub-Saharan Africa.

Table 3: Prevalence of sickle cell trait and disease in the Uganda EID program. From Kiyaga et al (123).

	US3		Targeted Screening Year 1		Targeted Screening Year 2		Targeted Screening Year 3		Total	
	Trait	Disease	Trait	Disease	Trait	Disease	Trait	Disease	Trait	Disease
<b>Central 1</b>	1896 (12.85)	69 (0.47)	417 (13.18)	34 (1.07)	1001 (13.42)	56 (0.75)	1079 (12.80)	54 (0.64)	4393 (12.99)	213 (0.63)
<b>Central 2</b>	1566 (14.06)	94 (0.84)	396 (14.75)	33 (1.23)	582 (14.68)	52 (1.31)	668 (13.97)	43 (0.90)	3212 (14.24)	222 (0.98)
<b>East Central Kampala</b>	1306 (19.85)	96 (1.46)	402 (19.67)	81 (3.96)	820 (18.90)	75 (1.73)	906 (19.37)	69 (1.47)	3434 (19.46)	321 (1.82)
	1835 (13.63)	90 (0.67)	733 (13.97)	79 (1.51)	1448 (14.12)	73 (0.71)	1352 (13.04)	83 (0.80)	5368 (13.65)	325 (0.83)
<b>Mid- Eastern</b>	752 (15.46)	60 (1.23)	221 (16.64)	43 (3.24)	417 (18.13)	41 (1.78)	366 (17.01)	31 (1.44)	1756 (16.50)	175 (1.64)
<b>Mid- Northern</b>	2445 (19.16)	160 (1.25)	1030 (18.59)	131 (2.36)	1418 (18.79)	130 (1.72)	1619 (18.27)	133 (1.50)	6512 (18.76)	554 (1.60)
<b>Mid- Western</b>	1431 (11.05)	64 (0.49)	121 (13.25)	16 (1.75)	334 (14.41)	18 (0.78)	393 (14.94)	25 (0.95)	2279 (12.12)	123 (0.65)
<b>North East</b>	702 (15.75)	46 (1.03)	165 (15.31)	22 (2.04)	495 (17.27)	49 (1.71)	440 (15.97)	29 (1.05)	1802 (16.15)	146 (1.31)
<b>South- Western</b>	631 (4.62)	23 (0.17)	21 (5.72)	2 (0.54)	56 (7.56)	0 (0.00)	58 (5.86)	1 (0.10)	766 (4.86)	26 (0.17)
<b>West Nile</b>	415 (13.76)	14 (0.46)	47 (16.04)	5 (1.71)	198 (17.38)	6 (0.53)	177 (15.83)	8 (0.10)	837 (15.04)	33 (0.59)
<b>TOTAL</b>	12979 (13.29)	716 (0.73)	3553 (15.68)	446 (1.97)	6769 (15.77)	500 (1.16)	7058 (15.09)	476 (1.02)	30359 (14.46)	2138 (1.02)

## Public Health Significance

Millions of people are affected by sickle cell disease, as it persists as one of the most common and lethal hereditary hematologic disorders in the world and is heavily concentrated in malaria endemic areas that are limited in resources and infrastructure. Sickle cell disease has adversely impacted these populations for thousands of years, yet its pathophysiology, diagnosis, and treatment remain elusive. Coupled with an already overwhelming and growing number of cases, sickle cell disease has become a neglected source of morbidity and mortality among children and older survivors in Africa. Sickle cell disease is overshadowed by well-publicized and well-funded infectious pandemics, though it may contribute up to 5% of under-five deaths on the African continent and 16% in individual high-prevalence countries,<sup>116</sup> which is comparable to the regional causes of childhood deaths from HIV at 4% and malaria at 15%.<sup>124</sup> High-income countries with low sickle cell disease prevalence experience survival and outcome success largely attributed to federal newborn screening. In contrast, in Africa, there are currently no institutional newborn screening programs and sickle cell disease related under-five mortality is estimated to be as high as 50-90%.<sup>46</sup>

The United Nations (UN),<sup>125</sup> WHO,<sup>46,116</sup> and African Union<sup>127</sup> have all declared sickle cell disease a major public health problem, particularly in sub-Saharan Africa, acknowledging the inadequate national sickle policies and plans, and making a call for interventions to identify the scope of the problem in each country and to address awareness, disease prevention, and early detection. The 2010 WHO report on sickle cell disease in the African region declared direct and

decisive priority interventions to address the burden of disease and posed the challenge that countries with high sickle cell disease prevalence develop and implement a national sickle cell disease control program within the context of their national health strategic plan by the year 2020. The current lack of policies and programs focused on sickle cell disease in these countries impedes their progress toward achieving United Nations Sustainable Development Goal 3, Good Health and Wellbeing, which aims to end preventable deaths of newborns and children under five years of age by 2030.<sup>128</sup>

Without early diagnosis and care, sickle cell disease is associated with a very high risk of death within the first five years of life. Many sub-Saharan African countries are experiencing an epidemiological shift where under-five mortality rates are decreasing due to improved nutrition, infectious disease control, and general child health services, but the relative contribution of genetic and other non-communicable conditions to early mortality is growing. The circumstance of higher allele frequencies and lower childhood mortality from other conditions means that sickle cell disease will occupy a larger proportion of deaths in children under-five, as well as an overall increase in sickle cell disease mortality due to limited access to care and treatment. The leading causes of under-five mortality in Sub-Saharan Africa are pneumonia, diarrhea, and other infections.<sup>124</sup> Co-morbidity with sickle cell disease contributes to poor prognosis in each of these conditions. The health and economic burden of sickle cell disease will increase proportionately and consume significant medical and social resources in high-burden low-resource settings. Only with proper diagnosis and treatment can the global inequity of the sickle cell disease burden be adequately recognized and managed.

## **SPECIFIC AIMS**

The overarching goal of this mixed-methods study was to conduct an epidemiological exploration of the novel national sickle cell screening program in Uganda.

**Aim 1: Characterize the epidemiology of sickle cell disease in Uganda.**

**Aim 2: Evaluate the centralized sickle cell screening laboratory in Uganda.**

**Aim 3: Describe healthcare providers' experiences with sickle cell screening in Uganda.**

## METHODS

### **Aim 1: Characterize the epidemiology of sickle cell disease in Uganda.**

#### *Study Design*

The study design was a cross-sectional quantitative analysis of sickle cell screening trends in Uganda over a five-year period, from February 2014 to March 2019, using the CPHL database.

#### *Study Setting*

The study setting was the CPHL, a unit under the National Disease Control Department of the Ministry of Health located in the capital city of Kampala. The CPHL provides laboratory support for disease surveillance, coordinates health laboratory services, assists in developing policy and guidelines, and conducts training and implementation of quality assurance schemes for laboratories throughout the country. The CPHL is home to the sickle cell laboratory that utilizes the National Sample Transport System and the CPHL database.

#### *Study Population*

The study population was children aged 0 to 24 months who were tested for sickle cell disease in the CPHL sickle cell laboratory from February 2014 to March 2019. The exclusion criteria

were children over the age of 24 months and who were tested for sickle cell disease in the CPHL sickle cell laboratory outside of routine sickle cell newborn screening, such as a community-testing event.

### *Data Collection*

Blood samples are routinely collected from infants by heel stick or finger stick on a standard dried blood spot card. The dried blood spot cards are labeled with the name of the health facility where the sample was collected, the date of collection, the infant's name and date of birth, and a unique identifying number. Each health facility completes a Dispatch Form with the demographic information transcribed from all of the dried blood spot cards collected, as well as each infant's address and telephone number for follow-up result reporting, and the testing service(s) being requested. US3 tested all samples requesting HIV testing for sickle cell disease. Sickle cell screening in high-burden districts includes all samples with a request for sickle cell disease testing, which includes samples that request both sickle cell disease and HIV testing or sickle cell disease testing only. The dried blood spot cards and associated Dispatch Form are packaged per established CPHL protocol and transported by motorbike to hubs at the sub district level and then shipped by courier to the CPHL in Kampala.<sup>117</sup>

When the samples are received at the CPHL, dried blood spots are examined to be deemed acceptable to move forward with testing. For accepted samples, all demographic information provided on the Dispatch Forms are entered into the CPHL database. Each dried blood spot card

has a barcode that is enacted to link the sample's unique identifying number with the demographic information in the database. The samples are then sent for testing per the request(s) on the Dispatch Form to respective laboratories.

In the CPHL sickle cell laboratory, IEF is performed on every sample accepted for sickle cell testing. Each sample is scored as normal, sickle cell trait, sickle cell disease, or variant. Variant hemoglobin is any hemoglobin that is not HbA, HbF, or HbS and was about 0.5% of samples in the US3 study. Two laboratory personnel independently enter preliminary results into the database where discrepancies are flagged and reviewed by a third laboratory personnel. Results are finalized in the database when any discrepancies are resolved. Result Report Forms for each sample tested for sickle cell disease are prepared and printed at the CPHL and disseminated back to the health facilities through the National Sample Transport System.

### *Data Analysis*

The data was requested and abstracted from the CPHL database for the period of February 2014 to March 2019. The variables included in the data abstraction are detailed in Table 4. Crude birthrate data was requested from the Uganda Bureau of Statistics.

Table 4: Description of variables extracted from CPHL database for Aim 1.

<b>Variable</b>	<b>Description</b>	<b>Data Type</b>	<b>Data Source</b>
<b>Facility Name</b>	Name of health facility where patient sample was collected	Categorical	Dispatch Form
<b>Level</b>	Level of health facility	Categorical	Dispatch Form
<b>Hub</b>	EID hub for health facility	Categorical	Dispatch Form
<b>Region</b>	Region in Uganda	Categorical	Dispatch Form
<b>District</b>	District in Uganda	Categorical	Dispatch Form
<b>Entry Point</b>	Clinics of entry into health facility	Categorical	Dispatch Form
<b>Sex</b>	Patient sex	Categorical	Dispatch Form
<b>Age in Months</b>	Patient age in months	Continuous	Dispatch Form
<b>Date Dispatched</b>	Date patient sample dispatched from CPHL, to be considered the date of diagnosis	Date	Dispatch Form
<b>Sickle Cell Result</b>	Result from sickle cell laboratory	Categorical	Laboratory Entry
<b>EID Result</b>	Result from EID laboratory	Categorical	Laboratory Entry

Descriptive statistical analysis was conducted to describe the study population using counts, proportions, means, and standard deviations. A description of the total number of children program year was done and further stratified by sickle cell result (sickle cell disease, sickle cell trait, normal, or variant). Age-adjustment by program year was done. Geographic-adjustment was done by region and by district. The expected number of sickle cell trait and disease births was calculated by multiplying the sickle cell trait and disease prevalence rates by the crude birthrate for each district for each year. The percentage of coverage of sickle cell newborn

screening in high-burden districts was determined. The data was analyzed using Stata statistical software.<sup>130</sup>

## **Aim 2: Evaluate the centralized sickle cell screening laboratory in Uganda.**

### *Study Design*

The study design was an evaluation of the national central sickle cell laboratory in Uganda over a five-year period, from February 2014 to March 2019, using the CPHL sickle cell laboratory database and supplemental documents from the CPHL and Cincinnati Children's Hospital.

### *Study Setting*

The study setting is described above in Methods for Aim 1.

### *Study Population*

The study population is described above in Methods for Aim 1.

### *Data Collection*

The data collection is described above in Methods for Aim 1.

## Data Analysis

The data was requested and abstracted from the CPHL database for the period of February 2015 to March 2019. The variables included in the data abstraction are detailed in Table 5.

Supplemental data was requested from the CPHL and Cincinnati Children's Hospital.

Table 5: Description of variables extracted from CPHL database for Aim 2.

Variable	Description	Data Type	Data Source
<b>Facility Name</b>	Name of health facility where patient sample was collected	Categorical	Dispatch Form
<b>Level</b>	Level of health facility	Categorical	Dispatch Form
<b>Hub</b>	EID hub for health facility	Categorical	Dispatch Form
<b>Region</b>	Region in Uganda	Categorical	Dispatch Form
<b>District</b>	District in Uganda	Categorical	Dispatch Form
<b>Entry Point</b>	Clinics of entry into health facility	Categorical	Dispatch Form
<b>Status</b>	Status of participant sample for testing at intake at CPHL	Categorical	Dispatch Form
<b>Date Collected</b>	Date patient sample was taken at health facility	Date	Dispatch Form
<b>Date Received</b>	Date patient sample was received at CPHL	Date	Dispatch Form
<b>Date Tested</b>	Date patient sample tested in sickle cell laboratory	Date	Dispatch Form
<b>Date Dispatched</b>	Date patient sample dispatched from CPHL, to be considered date of diagnosis	Date	Dispatch Form
<b>Sickle Cell Result</b>	Result from sickle cell laboratory	Categorical	Lab Entry
<b>EID Result</b>	Result from EID laboratory	Categorical	Lab Entry

Sample volume was presented as counts and percentages and turnaround time was calculated in days. Sample volume was determined by month and year. Sample turnaround times were calculated from date of collection, receipt, tested, and dispatched. The data was analyzed using Stata statistical software.<sup>130</sup>

The cost of sickle cell screening was determined using information from procurement documents for equipment, reagents, consumables, and payroll and employee documents.

**Aim 3: Describe healthcare providers' experiences with sickle cell screening in Uganda.**

*Study Design*

The study design was qualitative using semi-structured interviews to describe healthcare provider' experiences with sickle cell screening at health facilities of different levels in high-burden districts in Uganda.

*Study Setting*

The study setting was three levels of health facilities sampled from high-burden districts in Uganda.

The structure of Uganda's healthcare system is a hierarchy of health facilities organized by size of the catchment population and available services. Health facilities that offer tertiary care include national referral hospitals and regional referral hospitals. Primary care health facilities include district hospitals, health center IV, health center III, health center II, and village/community health teams. The healthcare system works on a referral basis; if a health center II cannot handle a case, the case is referred to a health facility the next level up (health center III).

The catchment population for a health center III is about 30,000 people, about 100,000 people for health facility IV, and about 300,000 to 500,000 people for a district hospital.

Each health facility level has a defined staffing standard with clinical/technical teams often headed by medical officers at district hospitals and health center IV, and clinical officers or nursing officers at health center III.

Tertiary health facilities are operated directly by the central government, while primary care health facilities are operated by district governments that oversee planning and provision of services to adhere to the policies and standards of care set by the central government.

### *Study Population*

The study population included healthcare providers of sickle cell patients from health facilities of different levels in high-burden districts in Uganda, including heads of sickle cell clinical team, nurses, midwives, social workers, and laboratory personnel. These are key positions in the direct provision of sickle cell screening. There are various job titles at health facilities for these key positions, which are detailed in Table 6.

Table 6: Sickle cell healthcare provider categories and expected job titles to be interviewed during the study.

<b>Healthcare Provider Category</b>	<b>Job Titles</b>
Head of Sickle Cell Clinical Team	Medical Officer, Clinical Officer, Nursing Officer
Laboratory Personnel	Laboratory Technician, Laboratory Technologist
Other Personnel	Nurse, Midwife, Social Worker

#### *Sampling, Sample Size, and Recruitment*

The Mid Northern and East Central are regions with the highest burden of sickle cell trait and disease. Within these regions, high-burden districts have been the focus of the national sickle cell screening program. In the Mid Northern, Lira was selected based on the district's sizable population and high sickle cell trait and disease prevalence at approximately 20% and 1%, respectively. In the East Central, Jinja district was selected due to its large population and high estimated sickle cell trait prevalence of about 20% and disease prevalence of about 1.7%.

Within Lira and Jinja districts, three different healthcare facility levels were represented in the sample, including one regional referral hospital, one health center IV, and one health center III. Health center II was not included because screening services are mostly offered at health center III level and above. For health center IV and health center III within each district, one facility at each of these levels was selected based on the criteria of high-volume and high-burden. In total, two regional referral hospitals, two district hospitals, two health center IV, and two health center III were included in the sample.

In Lira and Jinja districts, up to five key positions were interviewed at each selected health facility within each level, including heads of sickle cell clinical team, nurses, midwives, social workers, and laboratory personnel. 23 semi-structured interviews were conducted.

### *Data Collection*

A semi-structured interview guide was developed to assess how, when, where and by whom sickle cell screening services are provided at the health facility; the process of sample collection and result reporting; and support and training received. The guide also assessed any issues of sickle cell screening among healthcare providers and caregivers, such as acceptability and ethical scenarios; perceived challenges of sickle cell screening and resolutions or proposals for improvement. The guide included probes for the study participant's experiences and observations at the health facility where they work (Appendix A).

The interviews were conducted face-to-face by study staff. Prior to the interview, the study staff provided the study participant with the informed consent document (Appendix B). The study staff and study participant reviewed and completed the informed consent with a date and signature. Each interview had an audio recording and handwritten field notes by study staff.

To ensure validity and reliability of study findings, study staff underwent training in conducting qualitative interviews; with a preference in selecting study staff who had qualitative research experience. During the training, the content, flow, clarity, and acceptability of the interview guide was assessed and no amendments were made.

### *Data Analysis*

A thematic content analysis was conducted in which text data were aggregated and assigned a code; similar codes were aggregated into themes using inductive (emergent) coding. With this approach, codes were derived from the data; codes were built and modified throughout the analysis process. All audio recordings were transcribed verbatim.

### **Human Subjects Considerations**

The original US3 protocol was approved by the School of Medicine Research Ethics Committee at Makerere University (SOMREC) and the Uganda National Council for Science and Technology

(UNCST) in Uganda. The study was also approved by the Cincinnati Children's Hospital Institutional Review Board and is funded by the Cincinnati Children's Research Foundation. The protocol is reviewed on an annual basis with approved amendments for additional research objectives. Amendments to reflect the objectives of this dissertation research, including consent documents and interview guides, were submitted and approved by SOMREC. Further approval was obtained and received from the University Of Texas School Of Public Health.

The Uganda Ministry of Health provided a waiver of informed consent for all US3 samples, which made it possible to repurpose dried blood spot cards. The waiver of consent has been extended to the ongoing sickle cell screening program.

## JOURNAL ARTICLE ONE

Trends in Sickle Cell Trait and Disease Screening in the Republic of Uganda, 2014-2019

*International Journal of Epidemiology*

### Introduction

Sickle cell anemia persists as one of the most common and deadly hereditary hematological disorders in the world caused by a homozygous mutation in the beta-globin gene.<sup>133</sup> The point mutation responsible for sickle cell arose thousands of years ago on at least four different occasions with origins in sub-Saharan Africa, India, and the Arab peninsula.<sup>6</sup> The mutation endured and expanded over generations due to the genetic selective advantage afforded by a single copy of the mutation against the threat of *Plasmodium falciparum* malaria. Yet for homozygotes, the sickle mutation was lethal early in life, rendering the sickle allele a balanced genetic polymorphism. Today, millions of people around the globe have inherited the sickle cell mutation and those carriers give birth to more than 300 000 children each year with sickle cell anemia, an already staggering number that is expected to increase by 30% over the next thirty years.<sup>7,8</sup>

The public health burden of sickle cell anemia is immensely disproportionate, as it is heavily concentrated in malaria endemic areas across much of sub-Saharan Africa and in other parts of the world that are limited in both resources and infrastructure. The lack of recognition and

inability to adequately manage patients with the disease in these settings leads to the rapid consumption of significant burden on medical and economic resources. High-income countries with low sickle cell anemia prevalence experience impressive survival success of affected infants, which is largely attributed to federal newborn screening.<sup>34,69,122</sup> In sub-Saharan Africa, where 75% of the annual global sickle cell anemia births occur,<sup>11</sup> there are currently no institutional newborn screening programs, resulting in affected children going undetected and dying at a very young age without any knowledge of their underlying condition. This underscores the need for early diagnosis of sickle cell anemia in high-burden countries to deliver the basic need of care and treatment to the greatest number of people suffering from the disease.

Uganda has been touted as a country with one of the highest burdens of sickle cell anemia in the world.<sup>12</sup> In 2013, the Uganda Ministry of Health endeavored to quantify the situation within its borders by conducting an epidemiological study of sickle cell trait and disease nationwide. The Uganda Sickle Surveillance Study (US3) documented a high national prevalence of sickle cell trait and disease of 13.3% and 0.7%, respectively.<sup>118</sup> These data provided detail information of the disease at the regional and even district level, documenting a non-uniform distribution across the country. Sickle cell trait was present in all districts, with prevalence that ranged from 2.5% up to 23.9%.<sup>118</sup> Many of the high-burden districts, with >20% sickle cell trait prevalence, were situated in the East Central and Mid Northern regions of Uganda. US3 became the first national-level study of its kind for sickle cell trait and disease in Africa, informing next-step screening strategies to address this burden.

Newborn sickle cell screening is relatively new in sub-Saharan Africa with only pilot studies conducted in a few countries including Angola,<sup>47</sup> Benin,<sup>48</sup> Ghana,<sup>49</sup> Democratic Republic of Congo,<sup>50</sup> Burkina Faso,<sup>51</sup> Tanzania,<sup>52</sup> Liberia,<sup>53</sup> and Nigeria.<sup>54</sup> Data from these local experiences have mounted evidence of the importance and feasibility of newborn sickle cell screening in Africa. This has highlighted the critical aspects of such programs, including capacity building for large-scale and quality testing that is cost-effective with the support from government, healthcare providers, and the community. While sickle cell disease strategies for Africa have been suggested by global governing bodies such as the United Nations,<sup>125</sup> the World Health Organization,<sup>46,116</sup> and the African Union,<sup>127</sup> there has been no universal consensus and deployment of a newborn sickle cell screening plan. This is likely due to the lack of information and analysis of large-scale long-term screening efforts in the region.

Here, we describe a detailed investigation of the ongoing sickle cell screening program in Uganda and offer information on screening results over the past five years. To document trends in sickle cell screening and provide insights on the utilization of this newly available service in Uganda, we examined sickle cell screening from 2014 to 2019, among newborns and young children up to 24 months of age.

## Methods

Uganda's sickle cell screening program is based at the Central Public Health Laboratories (CPHL), a unit under the National Disease Control Department of the Ministry of Health located in the capital city of Kampala. The CPHL's processes for data collection, testing, and result reporting have previously been described.<sup>117,118</sup>

From February 2014 to March 2015, US3 analyzed about 100 000 dried blood spots for sickle cell trait and disease, by co-testing all samples collected for HIV screening, as part of an integrated testing scheme with the Centers for Disease Control sponsored Early Infant HIV Detection (EID) program. The subsequent sickle cell screening program commenced in April 2015, directly following US3, and included all samples with a specific request for sickle cell testing, which was indicated in several different ways. On the healthcare provider side, samples could have a request for sickle cell testing only, or for both sickle cell and HIV testing, from any district. On the CPHL side, there was an internally automated scheduling of samples for sickle cell testing if received from high-burden districts, regardless of whether the original request was for sickle cell testing only, for both sickle cell and HIV testing, or HIV testing only. All samples found HIV-positive were also automatically sent for sickle cell testing.

The study population for this analysis included all children in Uganda, aged 0-24 months who underwent sickle cell screening by the CPHL from February 2014 to March 2019. The exclusion criteria were children over age 24 months, and those who were tested for sickle cell trait and disease at CPHL outside of routine sickle cell screening, such as a community-wide testing event.

## *Statistical Analysis*

Sickle cell screening was analyzed in two cohorts, based on how the testing service for sickle cell was requested. The sickle/HIV co-testing cohort included any sample in the database that had both a sickle cell result and HIV result. The sickle specific testing cohort included any sample that had only a sickle cell result.

For each cohort, the frequency of children testing as normal, sickle cell trait, sickle cell disease, or other hemoglobin variant were described as annual prevalence rates by program year. US3 was considered program year 1, followed by years 2 to 5 for each 12-month period through March 2019. Age-adjustment in five-month age groups was done for each cohort by program year. Screening proportions with cross-tabulation of sickle cell result and program year, sickle cell result and age, sickle cell result and region, and sickle cell result and districts were then performed. The expected number of sickle cell disease births was calculated by multiplying the sickle cell disease prevalence rates by the crude birthrate for each district in 2018. The coverage of sickle cell screening in high-burden districts was determined by dividing the number of sickle cell disease positive results by the expected sickle cell disease births within each district.

## Results

### *Population Screened*

Since the onset of sickle cell screening in Uganda in February 2014, a total of 324 356 samples were tested in the CPHL sickle cell laboratory. Most of the samples (91.5%) were collected before 24 months of age with a median age of 2 months (IQR 2-8 months). There was further exclusion of 15985 samples that were tested outside of routine CPHL newborn screening and 2113 samples outside the date range. The study population was balanced with respect to gender (females, 50.1%). The final analysis included 278 651 children screened (Figure 1).

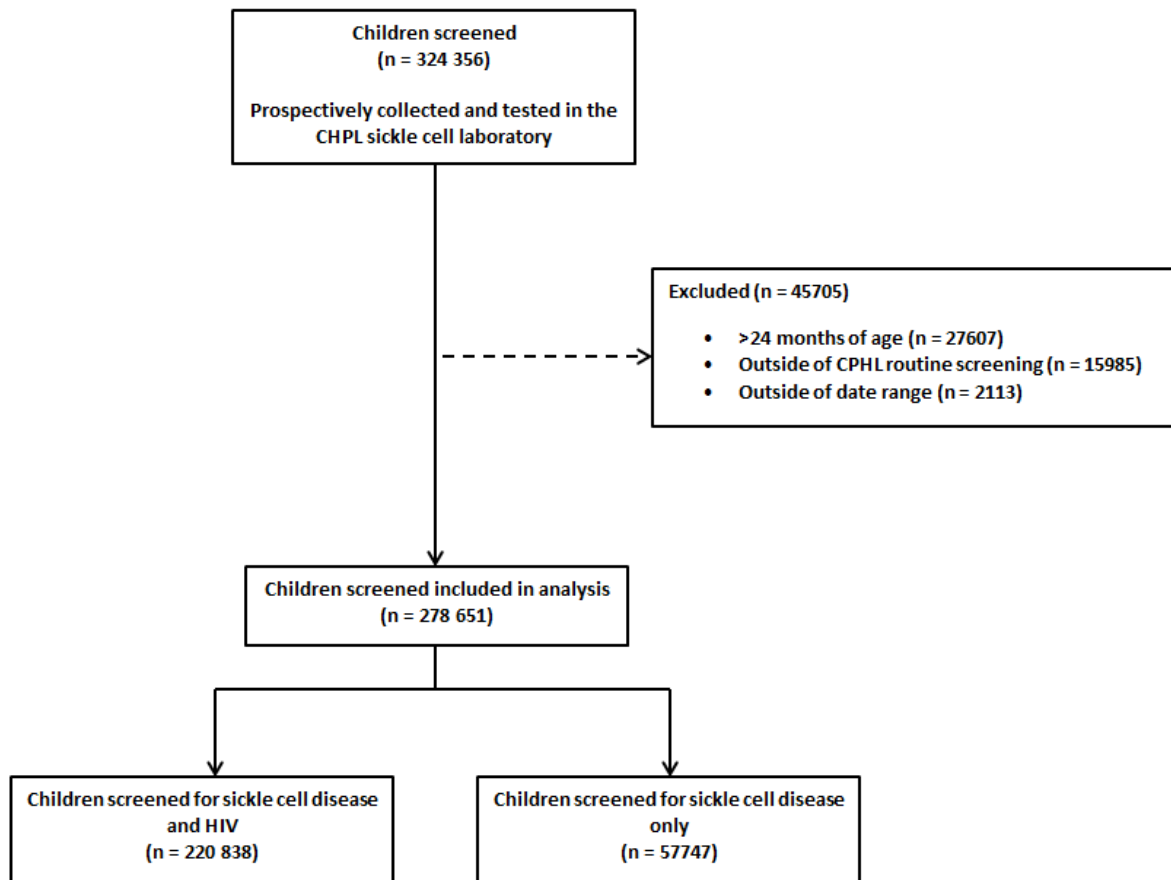


Figure 1: Flow chart of children in CPHL sickle cell screening included in final analysis.

Table 1 details testing services requested on the dispatch form by healthcare providers at health facilities or by an internal process within the CPHL for sickle cell screening. The sickle/HIV co-testing cohort included 79.3% (n = 220 838) of all children screened with median age of 2 months (IQR 2-8 months). Sickle specific testing comprised 20.7% (n = 57747) of screened children with median age of 1 month (IQR 0-7 months).

Table 1: Details of Uganda sickle cell screening cohorts

<b>Cohort</b>	<b>Request Type</b>	<b>Source</b>
Sickle/HIV Co-Testing	Sickle cell and HIV testing from any district	Healthcare providers at health facilities
	Sickle cell and HIV testing from high-burden districts	CPHL internal process
	HIV-positive from any district	CPHL internal process
Sickle Specific Testing	Sickle cell only testing from any district	Healthcare providers at health facilities

### *Prevalence of Sickle Cell Trait and Disease*

The prevalence of sickle cell trait and disease for both cohorts by program year are summarized in Table 2. Over five years, the sickle/HIV co-testing cohort had an overall sickle cell trait prevalence of 14.4% and disease prevalence of 0.9%, which is similar to the initial US3 screening results. For the sickle specific testing cohort, however, there was a large increase in the diagnosis of sickle cell disease after year 1 (0.7% to 9.7%). The overall prevalence of sickle cell trait and disease identified in the 5-year screening period was 14.7% and 2.8%, respectively (Table 1).

Table 2: Characteristics of children aged 0-24 months who underwent sickle cell screening in Uganda by program year.

Characteristics	Total Screened						Sickle/HIV Co-testing					Sickle Specific Testing				
	Year 1	Year 2	Year 3	Year 4	Year 5	Total	Year 2	Year 3	Year 4	Year 5	Total	Year 2	Year 3	Year 4	Year 5	Total
<b>Result (%)</b>																
<b>n</b>	98965	11415	56379	69511	42315	278 585	9100	43169	46918	22695	220 838	2315	13210	22593	19620	57747
<i>Normal</i>	84.1	79.5	80.1	80.8	78.3	81.4	81.9	82.2	83.3	83.4	83.4	70.2	73.5	75.7	72.4	73.8
<i>Sickle Cell Trait</i>	13.1	16.3	15.9	15.0	15.8	14.7	16.2	15.8	15.1	15.1	14.4	16.8	16.4	14.7	16.6	15.8
<i>Sickle Cell Disease</i>	0.7	3.3	3.1	3.6	5.4	2.8	1.1	1.2	1.0	1.1	0.9	12.1	9.4	9.0	10.5	9.7
<i>Other Variant</i>	0.5	0.9	0.9	0.6	0.5	0.6	0.9	0.9	0.6	0.4	0.6	0.8	0.8	0.6	0.5	0.6
<i>Invalid</i>	1.6	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0
<b>Age, months</b>																
<b>n</b>	98965	11415	56445	69511	42315	278 651	9100	43169	46918	22695	220 838	2315	13210	22593	19620	57747
<i>0-4</i>	62.0	69.2	70.2	71.4	66.9	67.1	72.3	71.8	71.3	65.4	66.7	57.2	65.0	71.7	68.6	68.5
<i>5-9</i>	13.2	13.9	14.4	14.8	15.8	14.2	13.1	14.4	15.9	17.2	14.4	16.9	14.3	12.5	14.1	13.6
<i>10-14</i>	16.5	10.5	9.9	8.8	11.0	12.2	10.1	9.7	8.9	12.1	12.9	11.8	10.7	8.4	9.6	9.5
<i>15-19</i>	7.9	5.3	4.7	4.1	5.2	5.8	4.3	3.9	3.6	5.0	5.7	9.0	7.1	5.2	5.4	5.9
<i>20-24</i>	0.4	1.2	0.9	0.9	1.2	0.8	0.2	0.2	0.2	0.3	0.3	5.1	2.9	2.3	2.4	2.6
<b>Region %)</b>																
<b>n</b>	98965	11412	56445	69503	42315	278 640	9097	43169	46910	22695	220 827	2315	13210	22593	19620	57747
<i>Central 1</i>	15.2	14.2	14.4	13.8	11.3	14.0	16.7	17.4	18.0	16.2	16.4	4.3	4.5	5.0	5.6	5.0
<i>Central 2</i>	11.3	8.7	10.8	20.8	26.1	15.7	10.2	9.2	10.3	13.0	10.8	2.8	16.1	42.7	41.3	34.5
<i>East Central</i>	6.7	12.1	9.4	8.5	7.9	8.1	11.8	10.1	10.0	10.6	8.7	13.2	7.2	5.4	4.8	5.9
<i>Kampala</i>	13.9	24.7	28.8	20.1	20.1	19.9	22.5	23.9	22.1	18.2	18.4	33.7	45.2	16.0	22.3	25.5
<i>Mid Eastern</i>	5.0	5.2	4.6	3.7	3.6	4.4	5.4	5.4	4.6	5.3	5.0	4.3	2.3	1.7	1.7	1.9
<i>Mid Northern</i>	13.2	20.8	17.8	19.7	17.6	16.7	17.6	17.6	18.9	19.9	16.2	33.7	18.4	21.4	14.8	19.0
<i>Mid Western</i>	13.2	4.2	4.8	4.7	4.1	7.6	5.1	5.4	5.6	5.4	8.9	0.7	2.9	2.9	2.7	2.7
<i>North East</i>	4.5	7.0	5.9	5.5	6.1	5.4	7.1	6.7	5.9	6.1	5.5	6.8	3.2	4.6	6.2	4.9
<i>South Western</i>	13.9	1.4	1.4	1.5	1.9	5.9	1.6	1.7	2.1	3.3	7.4	0.4	0.2	0.1	0.3	0.2
<i>West Nile</i>	3.1	1.7	2.1	1.7	1.3	2.2	2.1	2.7	2.4	2.0	2.7	0.0	0.1	0.1	0.4	0.2

### *Age-Adjusted Sickle Cell Screening Trends*

The age at which children were screened in both cohorts was predominantly in the youngest age group of 0-4 months, comprising 66.7% of the sickle/HIV co-testing cohort and 68.5% of the sickle specific cohort (Table 2). There was a progressive decrease in the proportion of children screened with increased age, although the sickle specific cohort included more children in the older age groups of 16-20 months and 21-24 months, in comparison to the sickle/HIV co-testing cohort. The sickle specific cohort also identified more children who screened sickle cell disease positive within the age range of 5-9 months. In contrast, the sickle/HIV co-testing cohort identified more disease within the younger age group of 0-4 months (Figure 2).

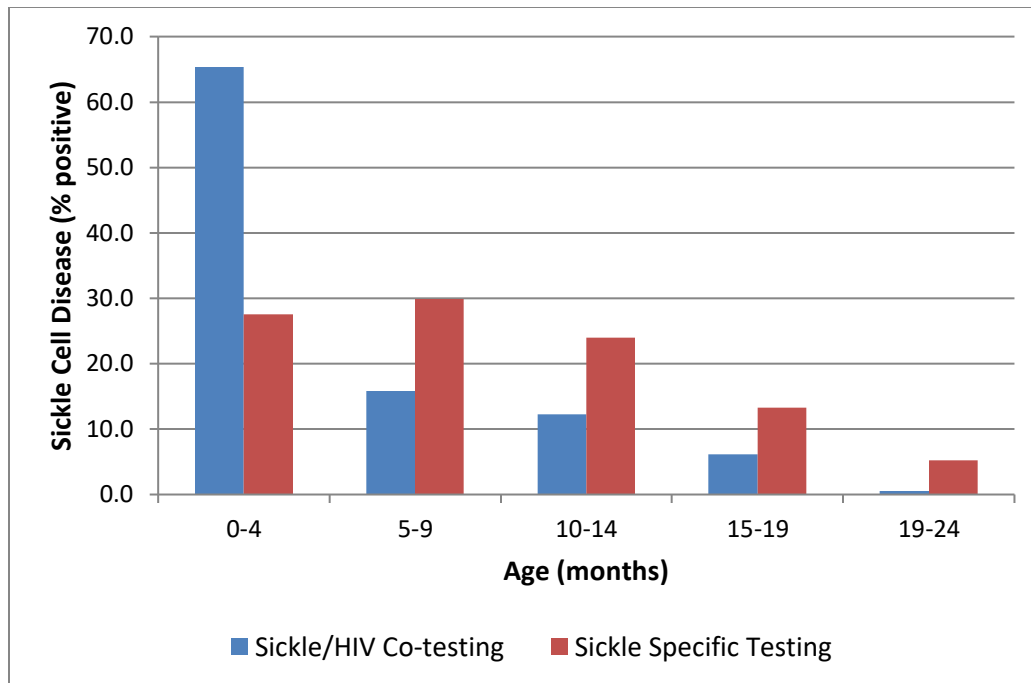


Figure 2: Proportion of patients screened that tested positive for sickle cell disease based on age group.

### *Geographic Sickle Cell Screening Trends*

Over the five-year screening period, the prevalence of sickle cell trait and disease in the sickle/HIV co-testing cohort documented the highest burden in the East Central (19.5% and 1.6%) and Mid Northern (18.6% and 1.5%) regions (Table 3). In the sickle specific cohort, exceptionally high disease frequencies were observed in the same two regions. The sickle specific cohort identified almost triple the number of sickle cell disease patients (n = 5628) compared to the co-testing cohort (n = 2036).

Within the sickle specific cohort, the frequency of sickle cell disease ranged from 1.8% to 54% of samples tested in each district. The absolute numbers of sickle cell screening samples across these districts was highly variable as well. For example, Mukono district in the Central region screened only 4 patients, with 2 identified to have sickle cell disease, while Jinja district in the East Central Region had 30.5% (637 of 2086) of screened samples test positive for sickle cell disease.

Table 3: Total sickle cell trait and sickle cell disease results by region over a five-year period.

	Sickle/HIV Co-testing		Sickle Specific Testing	
	Sickle Cell Trait	Sickle Cell Disease	Sickle Cell Trait	Sickle Cell Disease
<b>Region n (%)</b>				
<i>Central 1</i>	4638 (12.8)	214 (0.6)	540 (18.5)	339 (11.6)
<i>Central 2</i>	3385 (14.2)	217 (0.9)	2511 (12.6)	961 (4.8)
<i>East Central</i>	3753 (19.5)	208 (1.6)	627 (18.4)	1068 (31.3)
<i>Kampala</i>	5482 (13.5)	296 (0.7)	2036 (13.8)	440 (3.0)
<i>Mid Eastern</i>	1844 (16.6)	147 (1.3)	210 (18.7)	401 (35.7)
<i>Mid Northern</i>	6626 (18.6)	527 (1.5)	2263 (20.6)	1316 (12.0)
<i>Mid Western</i>	2352 (12.0)	119 (0.6)	272 (17.2)	219 (13.9)
<i>North East</i>	1968 (16.2)	148 (1.2)	612 (21.6)	831 (29.3)
<i>South Western</i>	791 (4.8)	28 (0.2)	18 (13.7)	20 (15.3)
<i>West Nile</i>	895 (15.0)	32 (0.5)	38 (31.4)	33 (27.3)
<b>Total</b>	<b>31735 (14.4)</b>	<b>2036 (0.9)</b>	<b>9127 (15.8)</b>	<b>5628 (9.7)</b>

### *Screening Performance Among High-burden Districts*

Using annual crude birth rate data from the Uganda Bureau of Statistics for each district, an estimation of sickle cell disease births for high-burden districts and sickle cell screening coverage was determined (Table 4). Screening in several districts achieved high coverage of sickle cell disease births, such as Soroti district with 194 documented sickle cell disease births out of 294 estimated sickle cell disease births (65.9%), with almost all of these (96.9%) identified within the sickle specific cohort. Similarly, Lira and Luwero districts had almost 50% coverage of estimated affected births; both had a large number of sickle cell disease births (>200) and >90% were found by sickle specific screening. Jinja, Buikwe, and Kamuli captured about 30% of affected births, with Jinja specifically identifying 155 of its estimated 504 annual sickle cell disease births, 87.1% of which were found in the sickle specific cohort.

Table 4: Screening coverage of sickle cell disease births among high-burden districts in 2018.

District	Crude Birth Rate	Estimated Sickle Cell Disease Births	Sickle Cell Disease Screened	Sickle Specific Testing
	n	n	n (%)	n (%)
Wakiso	135780	909	53 (5.8)	40 (75.5)
Apac	36250	852	56 (6.6)	46 (82.1)
Kampala	89650	773	182 (23.6)	133 (73.1)
Jinja	22640	504	155 (30.8)	135 (87.1)
Iganga	24230	484	8 (1.7)	2 (25.0)
Torororo	21970	390	67 (17.2)	56 (83.6)
Butaleja	12920	332	12 (3.6)	12 (100.0)
Dokolo	10210	319	61 (19.1)	53 (86.9)
Mayuge	24300	316	34 (10.8)	29 (85.3)
Busia	16940	303	22 (7.3)	13 (59.1)
Soroti	16300	294	194 (65.9)	188 (96.9)
Lira	18190	293	136 (46.4)	126 (92.6)
Kole	11450	271	8 (3.0)	5 (62.5)
Bundibugyo	11340	256	30 (11.71)	29 (96.67)
Bugiri	16170	254	20 (7.9)	14 (70.0)
Oyam	17380	243	25 (10.3)	17 (68.0)
Luwero	20550	242	116 (48.0)	115 (99.1)
Buikwe	19310	238	78 (32.8)	69 (88.5)
Kamuli	17280	236	80 (33.8)	72 (90.0)
Kaliro	12080	227	5 (2.2)	2 (40.0)
Kibuku	12150	226	28 (12.4)	27 (96.4)
Albetong	11170	223	54 (24.3)	48 (88.9)
Kayunga	15300	211	57 (27.1)	52 (91.2)
Serere	12290	207	28 (13.5)	28 (100.0)
Buyende	12510	201	23 (11.4)	21 (91.3)
Luuka	10810	199	15 (7.6)	14 (93.3)

Pallisa	9440	183	1 (0.5)	1 (100.0)
Gulu	13940	169	35 (20.7)	27 (77.1)
Namayingo	9720	167	10 (6.0)	5 (50.0)
Pader	7430	159	25 (15.7)	20 (80.0)
Amuria	10660	153	21 (13.7)	21 (100.0)
Namutumba	11050	143	25 (17.5)	21 (84.0)
Kaberamaido	9460	134	33 (24.7)	29 (87.9)
Mubende	28930	131	10 (7.6)	6 (60.0)
Masindi	12090	125	9 (7.2)	7 (77.8)
Nebbi	8700	124	7 (5.7)	6 (85.7)
Budaka	10690	119	4 (3.3)	3 (75.0)
Kumi	10650	118	91 (77.1)	90 (98.9)
Agago	9700	110	2 (1.8)	2 (100.0)
Nwoya	7970	109	10 (9.2)	8 (80.0)
Mityana	13200	101	128 (127.0)	127 (99.2)
Buliisa	6330	100	8 (8.0)	4 (50.0)
Kitgum	7490	96	40 (41.6)	32 (80.0)
Abim	5130	91	13 (14.3)	9 (69.2)
Amuru	8270	75	16 (21.2)	13 (81.3)
Katakwi	5970	67	10 (14.8)	8 (80.0)
Amoltar	6160	65	52 (79.7)	49 (94.2)
Masaka	12900	60	34 (56.9)	28 (82.4)
Otuke	5590	59	13 (22.1)	12 (92.3)
Ngora	7090	59	34 (57.9)	33 (97.1)
Lamwo	5560	48	20 (41.8)	15 (75.0)
Nakasongola	6870	47	24 (51.4)	23 (95.8)
Rakai	9870	45	10 (22.2)	5 (50.0)
Adjumani	10390	39	12 (30.7)	12 (100.0)
Koboko	7760	16	1 (6.2)	0 (0.0)

Total	908180	11918	2249 (18.8)	1936 (87.4)
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## Discussion

US3 was a landmark study for Uganda that contributed knowledge, capacity, and awareness about sickle cell disease, a genetic disorder with enormous morbidity and early childhood mortality. For the past five years, from 2014 to 2019, more than 300 000 infants were tested for sickle cell trait and disease as part of an ongoing screening effort focusing on high-burden areas informed by US3. The burden of sickle cell disease in Uganda is immense and compelling from both an epidemiological and public health standpoint.

In the sickle/HIV co-testing cohort, representing children who were screened for sickle cell disease solely by having maternal HIV exposure, the sickle cell trait and disease frequencies were predictably consistent over five years, with the slight increase in year 2 likely due to focused screening in high-burden districts from that year forward. Analyses of these co-testing samples over time confirmed the high burden of sickle cell trait and disease in the country, and the results are consistent with the initial surveillance data from US3.<sup>46,123</sup> In contrast, the sickle specific cohort had a remarkably high sickle cell disease frequency, approaching 10% of samples tested (Table 2). These results reflect an increased knowledge and efficiency for sickle cell screening by local healthcare providers, presumably an expected result of sickle cell education and screening training efforts within the high-burden districts.

The observation of more sickle cell disease specific testing in the two older age groups (15-19 and 20-24 months) implies that healthcare providers are utilizing the national sickle cell

screening service to test children with signs and symptoms of disease presentation, as well as testing older patients up to 2 years who were suspected to have sickle cell disease before screening was available. Concurrently, the number of patients tested within the younger age groups of 0-4 months and 5-9 months increased over time, indicating that screening eventually shifted to identifying new patients at an earlier age.

In the sickle specific cohort, the largest proportion of children screened positive for sickle cell disease was in the age group of 5-9 months. Sickle cell disease typically presents with symptoms around 6 months of age including pallor, infections, dactylitis and other vaso-occlusive pain events; therefore, this peak testing age reflects the appropriate emphasis on testing patients suspected of having sickle cell disease by healthcare providers. The co-testing cohort mostly screened the same newborns as the EID program, with the vast majority tested before age 6 months (Table 1).

Analysis of sickle cell result by district from the sickle specific cohort showed considerable differences among districts, which raises questions about how healthcare providers are utilizing the sickle cell screening service at their health facilities, and specifically how they are selecting patients for screening. It is anticipated that a composite index of suspicion for sickle cell disease exists, including observation of clinical signs and symptoms and a family history of sickle cell trait or disease that warrants testing. Specific policies and processes at each district and down to the health facility level may also dictate how screening is accomplished, since there are no published national guidelines for sickle cell screening currently in place. Furthermore,

investigation of the screening coverage unveiled the occurrence of both stronger and weaker performance sites for sickle cell screening among the high-burden districts. This observation prompts the need to examine the experiences of these districts, to design and carry out interventions based on effective practices that already exist and to focus on areas for improvement across districts and health facility levels.

### *Strengths and Limitations*

This study provides an inaugural examination of trends from a novel national sickle cell screening program in Uganda. Five years of data were available, which provided a large sample size of about 325 000, as well as the ability to evaluate secular and geographic trends in screening. The ongoing screening provides surveillance by conveying a sense of a long-term vigil of the sickle cell burden in the country and can be used to evaluate interventions and policy implications. The current analysis highlights the success of the program as it transitioned from a preliminary research-based epidemiological survey to a focused screening approach, creating a high yield of sickle cell positive patients and effective screening coverage in selected high-burden districts. Screening shortcomings were also identified, however, such as some high-burden districts with relatively poor screening coverage.

The use of the CPHL centralized database was a major strength of this study. The results for all testing services associated with a single sample were available, therefore abstraction of the data for this study included both sickle cell and HIV results, which defined the sickle/HIV co-

testing and sickle specific testing cohorts. Sickle specific testing analysis was important because it gauged the sickle cell knowledge of healthcare providers and raised further questions about employing screening programs down to the health facility level. The CPHL database also provided demographic variables that enabled a detailed examination, including age of the child, and the region and district from which the sample was collected.

An important limitation related to the CPHL database was that grouping of the sickle/HIV co-testing cohort was based on having both results; however, further interrogation of the request type, whether initially requested by the healthcare provider or subsequently by a CPHL internal process, could not be done. Follow-up is the most important consequence of screening, therefore, the biggest limitation faced was the inability to ensure that all children identified with sickle cell disease in the study have received their result and have been entered into medical care. At CPHL, there are currently no further data available on samples beyond their test result, largely because health facilities do not have electronic medical records that can link this information back to CPHL.

### *Conclusion*

In Uganda, the strategy of focused sickle cell screening in high-burden districts has been very promising, based on the results of this study. Further investigation of the experiences of healthcare providers with sickle cell screening is needed, to detail the current utilization of this service and to refine practices with specific interventions. At this time, focused sickle cell

screening has been an effective and feasible approach to begin to confront the country's large burden of disease. However, as the adverse impact of sickle cell disease on the population becomes more fully realized, a shift toward earlier and more widespread screening will be most advantageous. The experiences in Uganda highlight the eventual goal of universal screening for all newborns, and the lessons learned to date are instructive for all countries in sub-Saharan Africa with a large sickle cell burden.

Supplementary Table: Sickle specific testing hemoglobin results by district.

District	Normal	Sickle Cell Trait	Variant	Sickle Cell Disease	Total	Normal	Sickle Cell Trait	Variant	Sickle Cell Disease
	n	n	n	n	n	%	%	%	%
Kampala	12164	2036	82	440	14722	82.6	13.8	0.6	3.0
Mityana	8366	1179	47	281	9873	84.7	11.9	0.5	2.8
Buikwe	5558	812	65	161	6596	84.3	12.3	1.0	2.4
Dokolo	1922	514	15	164	2615	73.5	19.7	0.6	6.3
Jinja	1065	376	8	637	2086	51.1	18.0	0.4	30.5
Mubende	1469	164	4	30	1667	88.1	9.8	0.2	1.8
Wakiso	1240	290	5	123	1658	74.8	17.5	0.3	7.4
Kitgum	1241	272	35	110	1658	74.8	16.4	2.1	6.6
Alebtong	924	274	6	111	1315	70.3	20.8	0.5	8.4
Gulu	827	324	11	110	1272	65.0	25.5	0.9	8.6
Lira	605	292	10	274	1181	51.2	24.7	0.8	23.2
Luweero	469	195	1	225	890	52.7	21.9	0.1	25.3
Kumi	424	210	6	248	888	47.7	23.6	0.7	27.9
Soroti	357	153	2	218	730	48.9	21.0	0.3	29.9
Omoro	464	154	6	81	705	65.8	21.8	0.9	11.5
Kamuli	299	85	3	169	556	53.8	15.3	0.5	30.4
Tororo	223	92	1	199	515	43.3	17.9	0.2	38.6
Lamwo	334	88	5	25	452	73.9	19.5	1.1	5.5
Hoima	325	65	7	27	424	76.7	15.3	1.7	6.4
Bundibugyo	246	93	0	82	421	58.4	22.1	0.0	19.5
Kayunga	174	62	0	156	392	44.4	15.8	0.0	39.8
Oyam	190	76	2	86	354	53.7	21.5	0.6	24.3
Butambala	267	46	0	33	346	77.2	13.3	0.0	9.5
Apac	179	72	1	85	337	53.1	21.4	0.3	25.2
Ngora	148	74	3	84	309	47.9	23.9	1.0	27.2
Mpigi	180	49	0	61	290	62.1	16.9	0.0	21.0
Masaka	145	86	1	51	283	51.2	30.4	0.4	18.0
Masindi	203	35	2	33	273	74.4	12.8	0.7	12.1
Mayuge	101	52	1	108	262	38.5	19.8	0.4	41.2
Otuke	147	44	1	47	239	61.5	18.4	0.4	19.7

Nakasongola	115	43	1	71	230	50.0	18.7	0.4	30.9
Serere	96	35	0	92	223	43.0	15.7	0.0	41.3
Amolatar	95	21	1	103	220	43.2	9.5	0.5	46.8
Kaberamaido	77	40	0	76	193	39.9	20.7	0.0	39.4
Nwoya	132	47	1	11	191	69.1	24.6	0.5	5.8
Kabarole	115	36	1	30	182	63.2	19.8	0.5	16.5
Buyende	104	34	0	24	162	64.2	21.0	0.0	14.8
Pader	64	31	0	54	149	43.0	20.8	0.0	36.2
Manafwa	82	27	1	24	134	61.2	20.1	0.7	17.9
Kibuku	37	24	0	72	133	27.8	18.0	0.0	54.1
Kole	75	24	2	31	132	56.8	18.2	1.5	23.5
Kotido	93	18	0	3	114	81.6	15.8	0.0	2.6
Amuria	47	20	1	41	109	43.1	18.3	0.9	37.6
Buvuma	78	24	0	6	108	72.2	22.2	0.0	5.6
Kalungu	47	27	0	28	102	46.1	26.5	0.0	27.5
Namutumba	34	17	1	42	94	36.2	18.1	1.1	44.7
Napak	35	26	0	31	92	38.0	28.3	0.0	33.7
Agago	69	16	2	4	91	75.8	17.6	2.2	4.4
Luuka	31	30	0	29	90	34.4	33.3	0.0	32.2
Busia	40	16	1	25	82	48.8	19.5	1.2	30.5
Mbale	36	28	1	17	82	43.9	34.1	1.2	20.7
Abim	50	12	0	17	79	63.3	15.2	0.0	21.5
Butaleja	21	11	2	35	69	30.4	15.9	2.9	50.7
Namayingo	23	13	2	23	61	37.7	21.3	3.3	37.7
Rakai	35	14	0	12	61	57.4	23.0	0.0	19.7
Buliisa	42	9	0	9	60	70.0	15.0	0.0	15.0
Nakaseke	35	10	0	12	57	61.4	17.5	0.0	21.1
Bugiri	24	6	1	23	54	44.4	11.1	1.9	42.6
Amuru	19	11	0	18	48	39.6	22.9	0.0	37.5
Kagadi	33	7	0	6	46	71.7	15.2	0.0	13.0
Kiboga	39	3	0	4	46	84.8	6.5	0.0	8.7
Adjumani	7	24	0	12	43	16.3	55.8	0.0	27.9
Mbarara	22	10	0	11	43	51.2	23.3	0.0	25.6
Ssembabule	39	1	0	2	42	92.9	2.4	0.0	4.8

Bukedea	18	15	0	6	39	46.2	38.5	0.0	15.4
Kalangala	16	8	0	14	38	42.1	21.1	0.0	36.8
Kiryandongo	13	11	0	13	37	35.1	29.7	0.0	35.1
Mukono	24	8	0	2	34	70.6	23.5	0.0	5.9
Nebbi	13	8	0	11	32	40.6	25.0	0.0	34.4
Kasese	23	3	0	6	32	71.9	9.4	0.0	18.8
Katakwi	15	4	1	11	31	48.4	12.9	3.2	35.5
Iganga	12	11	0	7	30	40.0	36.7	0.0	23.3
Bulambuli	17	3	0	9	29	58.6	10.3	0.0	31.0
Pallisa	14	3	0	11	28	50.0	10.7	0.0	39.3
Lyantonde	18	5	0	5	28	64.3	17.9	0.0	17.9
Buikwe	9	9	0	8	26	34.6	34.6	0.0	30.8
Lwengo	19	4	0	3	26	73.1	15.4	0.0	11.5
Kyenjojo	21	3	0	2	26	80.8	11.5	0.0	7.7
Gomba	15	4	0	4	23	65.2	17.4	0.0	17.4
Kibaale	13	3	0	5	21	61.9	14.3	0.0	23.8
Isingiro	15	1	0	2	18	83.3	5.6	0.0	11.1
Kyankwanzi	13	0	0	3	16	81.3	0.0	0.0	18.8
Budaka	7	2	0	6	15	46.7	13.3	0.0	40.0
Buhweju	10	4	0	1	15	66.7	26.7	0.0	6.7
Kaliro	8	1	0	5	14	57.1	7.1	0.0	35.7
Bukomansimbi	7	4	0	2	13	53.8	30.8	0.0	15.4
Bududa	10	2	0	1	13	76.9	15.4	0.0	7.7
Sironko	10	1	1	1	13	76.9	7.7	7.7	7.7
Koboko	6	1	0	4	11	54.5	9.1	0.0	36.4
Ntoroko	5	3	0	3	11	45.5	27.3	0.0	27.3
Arua	9	1	0	1	11	81.8	9.1	0.0	9.1
Rubirizi	8	0	0	2	10	80.0	0.0	0.0	20.0
Moroto	5	4	0	1	10	50.0	40.0	0.0	10.0
Rukungiri	8	1	0	1	10	80.0	10.0	0.0	10.0
Amudat	7	0	0	2	9	77.8	0.0	0.0	22.2
Kwania	4	3	0	2	9	44.4	33.3	0.0	22.2
Maracha Terego	4	2	0	2	8	50.0	25.0	0.0	25.0
Ibanda	6	1	0	1	8	75.0	12.5	0.0	12.5

Masindi	5	1	0	1	7	71.4	14.3	0.0	14.3
Kisoro	3	1	0	2	6	50.0	16.7	0.0	33.3
Kyegegwa	3	1	0	2	6	50.0	16.7	0.0	33.3
Namisindwa	5	0	0	1	6	83.3	0.0	0.0	16.7
Namutumba	3	2	0	1	6	50.0	33.3	0.0	16.7
Zombo	2	1	0	2	5	40.0	20.0	0.0	40.0
Mukono	0	2	0	2	4	0.0	50.0	0.0	50.0
Nakapiripirit	2	1	0	1	4	50.0	25.0	0.0	25.0
Pakwach	3	0	0	1	4	75.0	0.0	0.0	25.0
Kyotera	0	2	0	1	3	0.0	66.7	0.0	33.3
Kamwenge	14	1	0	0	15	93.3	6.7	0.0	0.0
Kakumiro	13	1	0	0	14	92.9	7.1	0.0	0.0
Kiruhura	7	0	0	0	7	100.0	0.0	0.0	0.0
Kaabong	4	0	2	0	6	66.7	0.0	33.3	0.0
Kanungu	4	0	0	0	4	100.0	0.0	0.0	0.0
Ntungamo	4	0	0	0	4	100.0	0.0	0.0	0.0
Yumbe	4	0	0	0	4	100.0	0.0	0.0	0.0
Kabale	3	0	0	0	3	100.0	0.0	0.0	0.0
Kapchorwa	2	1	0	0	3	66.7	33.3	0.0	0.0
Moyo	2	1	0	0	3	66.7	33.3	0.0	0.0
Bunyangabu	2	0	0	0	2	100.0	0.0	0.0	0.0
Kaabong	2	0	0	0	2	100.0	0.0	0.0	0.0
Bushenyi	1	0	0	0	1	100.0	0.0	0.0	0.0
Kween	1	0	0	0	1	100.0	0.0	0.0	0.0
Rubanda	1	0	0	0	1	100.0	0.0	0.0	0.0
Sheema	1	0	0	0	1	100.0	0.0	0.0	0.0
Total	42640	9127	352	5628	57747	73.8	15.8	0.6	9.7

## JOURNAL ARTICLE TWO

### Operational Analysis of the National Sickle Cell Screening Program in the Republic of Uganda

#### Introduction

Sickle cell anemia is a monogenic hematological disorder that manifests as a devastating systemic disease with high morbidity and early mortality. The pathophysiology begins in infancy with acute and life-threatening complications, which presents as increased susceptibility to infections, chronic hemolytic anemia, and painful vaso-occlusive events.<sup>5,133</sup> Sickle cell anemia is the most prevalent hemoglobinopathy that impacts more than 20 million people worldwide, with an estimated 312 302 babies born with the disease each year.<sup>11</sup> Over 75% of the world's annual sickle cell anemia births occur in sub-Saharan Africa where resources for providing early detection and care are most constrained,<sup>11</sup> leading to the deaths of more than 500 affected children per day.<sup>135</sup>

The World Health Organization (WHO) and other international groups have begun to recognize sickle cell anemia as a major public health concern around the globe, but particularly in sub-Saharan Africa where there is a dearth of government programs in place to address this overwhelming and growing burden of disease.<sup>46,116, 125,127</sup> These organizations have identified the need to implement affordable and evidence-based strategies that can be sustainably

integrated into existing healthcare systems, highlighting newborn screening as a priority intervention.<sup>46</sup>

Evidence from both high- and limited-resource countries has shown that newborn screening for sickle cell anemia can significantly decrease morbidity and mortality by enabling early initiation of penicillin prophylaxis and pneumococcal immunizations as primary prevention.<sup>69,122,134,136</sup> However, the accurate diagnosis of sickle cell anemia in limited-resource settings still faces major challenges related to high equipment and reagent costs, education and training of healthcare providers, and appropriate medical interventions. Newborn sickle cell screening requires efficient laboratory methodologies and infrastructure for early and accurate diagnosis to reduce preventable deaths among children born with sickle cell disease and to support the ultimate goal of universal screening of all babies across sub-Saharan Africa.

The Uganda Sickle Surveillance Study (US3) commenced in 2014 using a simple but innovative approach to screen children for sickle cell trait and disease on a national level.<sup>118</sup> Residual dried blood spots collected across the country as part of the Early Infant HIV Detection (EID) program were used. Isoelectric focusing (IEF) was conducted on about 100 000 samples over one year in a cross-sectional analysis and found an overall high prevalence of sickle cell trait at 13.1% and disease at 0.7%, but with a disproportionate distribution across the country. Broader sickle cell screening has been initiated since 2015, strategically focused on the highest-burden districts.

To expedite early detection and facilitate linkage to care for affected infants, it is crucial to identify where delays in diagnosis occur. Here, we describe a detailed operational analysis of the time and cost of sickle cell screening in Uganda. We documented the turnaround times (TAT) for sickle cell screening, starting from sample collection to arrival, testing, and result reporting at the national centralized laboratory. To examine the cost implications of integrated sickle cell screening, we calculated the cost per test, based on exact expenditures from US3, and made estimates of the cost-effectiveness of sickle cell screening in Uganda.

## Methods

### *Integrated Screening*

US3 was conducted from February 2014 to March 2015 to determine the prevalence of sickle cell trait and disease in Uganda. A foundational goal of the study was building local sickle cell laboratory capacity within the Ministry of Health's existing centralized laboratory infrastructure and determining the feasibility of high-volume sample through-put for scale-up to universal newborn screening. At the Central Public Health Laboratories (CPHL) in Kampala, Uganda, a partnership with Cincinnati Children's Hospital Medical Center (CCHMC) led to construction of a new sickle cell laboratory that was outfitted with isoelectric focusing (IEF) equipment, and local personnel were recruited and trained on a standardized hemoglobin electrophoresis protocol.

As previously described for the EID program, blood was collected from HIV-exposed infants and young children across the country using standard dried blood spot cards.<sup>117</sup> Each health facility populated a dispatch form with demographic information and testing requests. Samples were transferred to the CPHL via the national sample transport system.<sup>117</sup> In US3, all samples requesting HIV testing were also queued for sickle cell testing. The results were entered into a centralized database and disseminated back to health facilities and onward to caretakers. Following US3, the dispatch form included an option for sickle cell testing only.

#### *Turnaround time Analysis (TAT)*

The CPHL database collects laboratory data on all samples received and processed; data for this analysis were abstracted for children aged 0-24 months who underwent routine sickle cell screening over a five-year period from February 1, 2014 to March 31, 2019.

Inbound TAT was defined as the total number of days between sample collection at the local health facilities and result dispatch from CPHL back to the providers. Three phases were defined within inbound TAT: (1) sample delivery phase TAT was the time between sample collection and receipt at the CPHL; (2) sample testing phase TAT was the time between receipt at the CPHL and date of testing; and (3) sample result reporting phase TAT was the time between testing and result dispatch from the CPHL. Outbound TAT, defined as the number of days between result dispatch and result receipt by the caretakers, was not assessed in this analysis because those data points are not currently collected by CPHL.

Median TATs were calculated for inbound TAT and for the three phases of sample delivery, sample testing, and sample result reporting. Summary statistics included medians, 25% and 75% interquartile ranges (IQR), and frequencies. Program year 1 was the time period of US3, followed by years 2 to 5 for each 12-month period through March 2019. There were two distinct cohorts for sickle cell screening, based on how the sickle cell testing service was requested. The sickle/HIV co-testing cohort included all samples in the database that had both sickle cell and HIV results. The sickle specific testing cohort included all samples that had a sickle cell result only.<sup>137</sup>

The Kruskal-Wallis (KW) test identified statistically significant difference in TAT across groups of phase-specific parameters. Sample delivery TAT, sample testing TAT, and sample result reporting TAT were continuous dependent variables. Parameters differed by phase since these individual TAT assess different parts of the inbound sample continuum. For the sample delivery phase TAT, independent categorical variables were region, health facility level, program year, year 5 collection month, and testing cohort. For the sample testing phase TAT, independent categorical variables were program year, year 5 testing month, and testing cohort. For the sample result reporting phase TAT, independent categorical variables were program year, year 5 dispatch month, and testing cohort. Analysis by month was conducted using only year 5 data (April 1, 2018 to March 31, 2019) to better understand recent temporal trends in TAT. Data were analyzed using STATA.<sup>130</sup>

### *Cost analysis*

This cost analysis of sickle cell screening presents cost per test by IEF and cost per case detected. Costs were assessed by detailed analysis of all US3 screening program expenditures, using actual procurement documents and vendor price sheets for IEF equipment, reagents, and consumables, plus CPHL labor costs, including salary and other employment costs. Costs that were shared with other CPHL programs and the health facilities were not included, such as the dried blood spot kits, sample transportation, and local healthcare provider salaries and screening-related training. Expenses incurred by CCHMC for travel and initial training of CPHL sickle cell laboratory personnel were also not included. Cost for equipment, reagents, and consumables were reported in United States Dollars (USD) according to actual costs using out-of-country and in-country vendors. For labor costs, annual salaries were expressed in Uganda Shillings (UGX) and converted to USD using the purchasing power parity exchange rate.<sup>138</sup> National sickle cell disease prevalence data and the calculated cost per test were used to generate the cost per sickle cell disease case detected, by region of Uganda.

### **Results**

#### *Sickle Cell Testing TAT*

Between February 1, 2014 and March 31, 2019, a total of 324 356 samples were collected and tested at the CPHL. Samples were excluded from analysis if they were outside the study date

range, beyond 24 months of age, or collected and tested outside of routine sickle cell screening.

The number of samples remaining for the final analysis were 278 651. In the subsequent analysis, only samples with present and plausible dates for collection, receipt, testing, or dispatch were included depending on the TAT being calculated. Accordingly, there were 265 766 samples included in the inbound TAT calculation, 276 521 samples for sample delivery TAT, 263 417 samples for sample testing TAT, and 267 325 samples for sample result reporting TAT.

The overall median inbound TAT from sample collection to results dispatch was 16 days (IQR: 11, 24) (Figure 1). The majority of the samples ( $n = 228\,684$ , ~86%) did not exceed an inbound TAT of one month. Program year 1 had the largest sample volume ( $n = 90872$ ), followed by almost 70000 samples in year 4. The South Western region had the shortest median inbound TAT of 13 days (IQR: 9, 20), while the Mid Western region had the longest TAT of 18 days (IQR: 12, 26). The sickle cell/HIV co-testing cohort had a slightly shorter inbound TAT than the sickle specific cohort of 15 days and 17 days, respectively (Table 1).

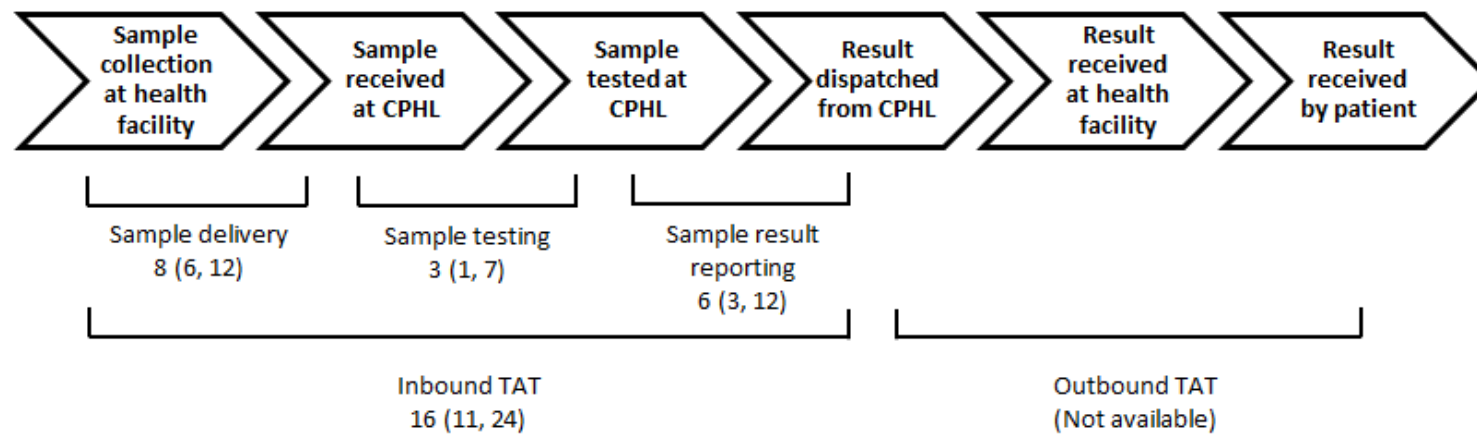


Figure 1: Central Public Health Laboratories sample continuum and turnaround time phases, with results shown as median in days (25%-75% IQR).

Table 1: Characteristics of sickle cell samples tested in Uganda from February 1, 2014 to March 31, 2019.

Characteristics	Inbound Samples <sup>a</sup>	Median Inbound TAT, days (IQR) <sup>b</sup>
<b>Program Year</b>	265 766	16 (11, 24)
<b>n</b>	265 766	
<i>Year 1</i>	90872	11 (8,15)
<i>Year 2</i>	8002	22 (17,29)
<i>Year 3</i>	56144	20 (15, 27)
<i>Year 4</i>	69052	21 (14, 33)
<i>Year 5</i>	41696	15 (11, 21)
<b>Region</b>		
<b>n</b>	265 755	
<i>Central 1</i>	37127	16 (11, 24)
<i>Central 2</i>	42188	17 (12, 25)
<i>East Central</i>	21550	17 (12, 26)
<i>Kampala</i>	53124	14 (9, 22)
<i>Mid Eastern</i>	1595	16 (11, 23)
<i>Mid Northern</i>	44730	18 (12, 26)
<i>Mid Western</i>	19959	15 (11, 22)
<i>North East</i>	14327	15 (10, 23)
<i>South Western</i>	15395	13 (9, 20)
<i>West Nile</i>	5760	15 (10, 23)
<b>Testing Cohort</b>		
<b>n</b>	265 708	
<i>Sickle Cell/HIV Co-Testing</i>	209 242	15 (10, 23)
<i>Sickle Specific Testing</i>	56466	17 (12, 25)

<sup>a</sup>12885 samples did not have available dates for collection and dispatch to calculate inbound TAT

<sup>b</sup>25%-75% IQR

For sample delivery phase, the median TAT was 8 days (IQR: 6, 12). Parameters affecting sample delivery TAT are shown in Table 2. The Kampala region, where the CPHL is located, had the shortest median sample delivery time of 6 days (IQR: 4, 9), while 6 regions had a median sample delivery time of 9 days. Regional referral hospitals had a shorter median sample delivery TAT of 7 days (IQR: 5, 9) compared to the lower health facility levels of health centers II, III, and IV. The collection month of December 2018 had the longest median TAT of 10 days (IQR: 5, 18) compared to other collection months. There was a statistically significant difference in sample delivery TAT among the 10 regions, health facility levels, program years, collection months for year 5, and the two testing cohorts (Table 2).

Table 2: Parameters affecting sample delivery phase turnaround time for sickle cell samples collected in Uganda from February 2014 to March 2019, n = 276 521.

Parameter		Samples Collected <sup>a</sup>	Sample Delivery TAT Median, days (IQR) <sup>b</sup>	p-value <sup>c</sup>
Region, n = 276 518	Central 1	38695	8 (6, 13)	0.0001
	Central 2	43494	9 (7, 13)	
	East Central	22464	9 (6, 13)	
	Kampala	55103	6 (4, 9)	
	Mid Eastern	12117	9 (6, 13)	
	Mid Northern	46333	9 (6, 13)	
	Mid Western	21044	9 (7, 13)	
	North East	14878	7 (5, 11)	
	South Western	16335	9 (6, 14)	
	West Nile	6047	8 (6, 12)	
Health Facility Level, n = 275 894	Regional Referral Hospital	17895	7 (5, 9)	0.0001
	Health Center IV	47033	8 (6, 13)	
	Health Center III	93816	9 (6, 14)	
	Health Center II	13675	9 (6, 13)	
	Hospital	72336	6 (6, 13)	
	Special Clinic	31139	6 (4, 9)	
Program Year, n = 276 521	Year 1	97782	8 (6,12)	0.0001
	Year 2	11414	9 (6,14)	
	Year 3	56203	8 (6,12)	
	Year 4	69100	9 (6, 13)	
	Year 5	42022	8 (5,12)	
Year 5 Collection Month, n = 40649	April 2018	5422	8 (4, 11)	0.0001
	May 2018	5163	7 (3,10)	
	June 2018	3263	8 (5, 12)	
	July 2018	3394	7 (4, 11)	

	August 2018	3361	8 (5, 12)	
	September 2018	3240	9 (6, 13)	
	October 2018	3371	8 (5, 12)	
	November 2018	3094	8 (5, 12)	
	December 2018	2309	10 (5, 18)	
	January 2019	3159	8 (5, 11)	
	February 2019	2825	9 (5, 13)	
	March 2019	2048	6 (3, 9)	
Testing Cohort, n = 276 457	Sickle/HIV Co-testing	219 156	8 (6, 12)	0.0001
	Sickle Specific Testing	57301	9 (6, 13)	

<sup>a</sup>2130 samples did not have available dates for collection and received to calculate sample delivery TAT

<sup>b</sup>25%-75% IQR

<sup>c</sup>a variable was significant when p-value  $\leq 0.05$

Once the sample arrived at the CPHL, the median TAT for sample testing was 3 days (IQR: 1, 7) and for sample result reporting was 6 days (IQR: 3, 12). For sample testing phase TAT, program year 4 had the longest median TAT of 7 days (IQR: 4, 14) (Table 3). Median sample testing and sample result reporting TATs were highest in the months of December 2018 and January 2019 (Tables 3 and 4). The two testing cohorts had similar median sample testing and sample result reporting TATs.

Table 3: Parameters affecting sample testing phase turnaround time for sickle cell samples collected in Uganda from February 2014 to March 2019, n = 263 417.

Parameter		Samples Tested <sup>a</sup>	Sample Testing TAT Median, days (IQR) <sup>b</sup>	p-value <sup>c</sup>
Program Year, n = 263 417	Year 1	94184	0 (0, 1)	0.0001
	Year 2	2157	4 (2, 5)	
	Year 3	55844	5 (3, 7)	
	Year 4	69377	7 (4, 14)	
	Year 5	41855	6 (4, 10)	
Year 5 Testing Month, n = 42201	April 2018	6157	4 (3, 6)	0.0001
	May 2018	5682	5 (4, 6)	
	June 2018	3443	4 (2, 6)	
	July 2018	3429	5 (3, 6)	
	August 2018	3436	5 (4, 7)	
	September 2018	3014	4 (3, 6)	
	October 2018	3365	5 (4, 8)	
	November 2018	3066	6 (5, 10)	
	December 2018	1706	12 (8, 13)	
	January 2019	3516	13 (9, 20)	
	February 2019	2604	11 (6, 13)	
	March 2019	2783	10 (8, 15)	
Testing Cohort, n = 263 400	Sickle Cell/HIV Co-testing	208 210	3 (0, 8)	0.0001
	Sickle Specific Testing	55190	4 (2, 6)	

<sup>a</sup>15234 samples did not have available dates for received and tested to calculate sample testing TAT

<sup>b</sup>25%-75% IQR

<sup>c</sup>a variable was significant when p-value  $\leq 0.05$

Table 4: Parameters affecting sample result reporting phase turnaround time for sickle cell samples collected in Uganda from February 2014 to March 2019, n = 267 325.

Parameter		Samples Dispatched <sup>a</sup>	Sample Result Reporting TAT Median, days (IQR) <sup>b</sup>	p-value <sup>c</sup>
Program Year, n = 267 325	Year 1	91645	2 (1, 3)	0.0001
	Year 2	8002	12 (8, 17)	
	Year 3	56381	10 (7, 15)	
	Year 4	69437	11 (6, 23)	
	Year 5	41860	6 (4, 10)	
Year 5 Dispatch Month, n = 42474	April 2018	6319	5 (3, 6)	0.0001
	May 2018	5720	5 (4, 7)	
	June 2018	3441	5 (3, 6)	
	July 2018	3455	5 (3, 6)	
	August 2018	3440	5 (4, 7)	
	September 2018	3056	4 (3, 7)	
	October 2018	3363	5 (4, 8)	
	November 2018	3072	6 (5, 10)	
	December 2018	1704	12 (8, 13)	
	January 2019	3517	13 (9, 20)	
	February 2019	2605	11 (6, 13)	
	March 2019	2782	10 (8, 15)	
Testing Cohort, n = 267 265	Sickle Cell/HIV Co-testing	210425	6 (2, 12)	0.0001
	Sickle Specific Testing	56840	6 (4, 12)	

<sup>a</sup>11326 samples did not have available dates for tested and dispatched to calculate sample result reporting TAT

<sup>b</sup>25%-75% IQR

<sup>c</sup>a variable was significant when p-value  $\leq 0.05$

### *Sickle Cell Screening Costs*

Inputs included in the cost analyses were exact direct expenditures for equipment, reagents, consumables, and labor for 99243 US3 samples tested by IEF from a dried blood spot at the CPHL (Table 5). The total equipment costs were annualized and calculated at \$0.94 per test. Reagent costs and consumables were calculated at \$1.04 and \$0.15 per test, respectively. Annual salary for laboratory personnel included gross pay, National Social Security Fund 10% monthly tax, customary “13<sup>th</sup> month” end-of-year compensation, and medical insurance per standard MOH employee contracts. Labor costs were \$2.33. The overall cost per test was \$4.46. Personnel costs made up more than half of the cost per test total at 52%, followed by reagents (23%), equipment (21%), and consumables (3%).

Table 5: Costs details for sickle cell screening and testing 99243 children over 25 months.

<b>Cost Category</b>	<b>Item (Qty)</b>	<b>Cost (USD)</b>
Equipment	Electrophoresis unit (4)	21600.00
	Water bath	5800.00
	Power supply (2)	11600.00
	Rocking platform	1200.00
	Gel dryer	670.00
	Puncher	15600.00
	Puncher computer	1900.00
	Puncher workstation	1900.00
	Puncher printer	165.00
	Glow box	964.00
	Replacement electrode (6)	3528.00
	Freezer	4400.00
	Refrigerator	6900.00
	Distiller	3145.00
	Value-added tax <sup>a</sup>	14340.96
	Equipment total	93712.96
	Equipment cost per test total	0.94
	<b>Item</b>	<b>Cost per Test (USD)</b>
Reagents	RESOLVE hemoglobin kit	0.83
	HbFASC control kit	0.06
	JB-2 stain solution kit	0.12
	Trichloroacetic acid	0.03
	Reagent cost per test total	1.04
	<b>Item</b>	<b>Cost per Test (USD)</b>
Consumables	96-well plates	0.07
	Gloves	0.08
	Pipette tips	0.01
	Consumables cost per test total	0.15
	<b>Personnel (Qty)</b>	<b>Annual Salary (USD)<sup>b</sup></b>
Labor	Laboratory manager	50056.01
	Laboratory technician (2)	86763.74
	Laboratory assistant	20022.40
	Data officer	33370.67
	Data clerk	20022.40
	Personnel other - medical insurance	20945.82
	Personnel total	231 181.05
	Labor cost per test total	2.33
<b>Cost per test total</b>		<b>4.46</b>

<sup>a</sup>Value-added tax for imported equipment

<sup>b</sup>2015 purchasing power parity exchange rate (\$1071.30) applied for conversion from UGX to USD

Using the calculated total cost per test and national prevalence data, cost per sickle cell disease case detected was calculated to be \$483.74 (Table 6). When stratified by region, however, the cost per case detected ranged widely from \$278.07 in the East Central region to \$2607.19 in the South Western region (Table 6).

Table 6: Cost per positive sickle cell disease case by region.

<b>Region</b>	<b>Sickle Cell Disease</b>	<b>Total Screened</b>	<b>Prevalence</b>	<b>Cost per Positive Case (USD)</b>
<i>Central 1</i>	214	36173	0.0059	753.89
<i>Central 2</i>	217	23872	0.0091	490.64
<i>East Central</i>	308	19203	0.0160	278.07
<i>Kampala</i>	296	40635	0.0073	612.27
<i>Mid Eastern</i>	147	11109	0.0132	337.05
<i>Mid Northern</i>	527	35671	0.0148	301.88
<i>Mid Western</i>	119	19686	0.0060	737.81
<i>North East</i>	148	12151	0.0122	366.17
<i>South Western</i>	28	16368	0.0017	2607.19
<i>West Nile</i>	32	5959	0.0054	830.54
<i>Total</i>	2036	220827	0.0092	483.74

## Discussion

For any newborn screening program, reduction of sample TAT is an important intervention to ensure that poor health outcomes or preventable deaths are not the result of operational laboratory or operational delays. Identification of the specific barriers related to longer TAT and implementation of measures to mitigate those causes are essential to strengthen screening programs.

In this detailed operational analysis of TAT for sickle cell screening in Uganda, the total duration between sample collection at the health facility to the dispatch of results from the CPHL averaged 16 days (Figure 1). Multiple parameters affected this time interval including health facility level, program year, collection month, and testing cohort. However, the vast majority (~86%) had results sent by CPHL back to the local healthcare facilities within one month of receipt, which we propose as the longest acceptable TAT before putting sickle cell disease patients at risk by delaying diagnosis and treatment.

For the Uganda sickle cell screening program, sample delivery consumed the most time for the inbound TAT (Figure 1). Lower health facilities had greater barriers to timely delivery, likely due to delayed collection of their samples for transport (Table 2). Within each district, hub motorbike riders perform a weekly pickup to the more rural locations of health centers II, III, and IV in comparison to regional referral hospitals or district hospitals. It is also possible that samples are not readily available for transport at the time the hub motorbike rider comes for

collection on an already intermittent basis. To address these issues, a routine schedule for sample pick-up with validation measures, such as time stamps, should be put into place. This should also be supplemented with ongoing training regarding sample preparation and the importance of timely sample transport for all personnel involved in the process.

After sample receipt at the CPHL, sample testing was relatively rapid with an average of only 3 days (Figure 1). This phase involves the routing of samples for sickle cell testing to the laboratory, punching and eluting dried blood spots, testing by IEF, and database result entry, therefore, this internal process can be deemed efficient. However, when looking at the parameter of program year, it does appear that longer TAT in this phase is associated with increased sample volumes (Table 3). This may be the cause of reagent stock-out or point to a need for more cross-training of CPHL personnel on the IEF procedure to support the sickle cell laboratory. As the program expands toward the eventual goal of universal newborn screening, more human resources will be necessary to handle the increased number of samples.

Following sample testing, result reporting took an average of 6 days (Figure 1). Altogether, the time that samples spent at the CPHL was prolonged. Once a sample is tested, issues in the interim to result dispatch could be due to stock-out of printing supplies. It was also observed that the testing and dispatch months of December 2018 and January 2019 had distinctly longer TATs (Table 4). This may be linked to personnel leave due to the holidays, therefore increasing or staggering support in the sickle cell laboratory during this time could help avoid lengthy delays.

In a study by Kiyaga et al at the CPHL regarding TAT of EID screening, the average sample delivery TAT was 12 days, sample processing was 2 days, and the overall average TAT from sample collection to reporting to the patient was 26 days.<sup>117</sup> As part of this study, short message service (SMS) printers were piloted to allow test results to be transmitted immediately to health care facilities after testing, which further reduced the overall TAT to 14 days.<sup>117</sup> In the US, the National Newborn Screening and Global Resource Center reported newborn bloodspot screening TAT to be within 10 to 14 days from sample collection, with variation by state.<sup>139</sup> To overcome these differences in state programs, the US Department of Health and Human Services (HHS) Advisory Committee on Heritable Disorders in Newborns and Children recently set out updated newborn screening timeliness goals specifying that tests should be completed within 7 days of birth.<sup>140</sup> In our study, we found that TAT for sickle cell screening was shorter in all inbound phases compared to what was previously reported for Uganda's EID program, exhibiting overall improved time efficiency for the CPHL sample continuum. The current program is also meeting a comparable timeframe to that of the long-standing US newborn screening program and will likely continue to improve with time and experience.

Information on outbound TAT could not be examined in this study, because those data are currently not collected by the CPHL. However, with greater visibility and a push toward newborn screening in sub-Saharan African countries, such as by the new American Society of Hematology African Screening Consortium,<sup>141</sup> outbound TAT and individual patient follow-up data will be coveted information. We predict that TAT will greatly impact healthcare provider

and patient utilization of sickle cell screening, as well as overall satisfaction with the program in Uganda. Most importantly, prolonged TAT very likely postpones life-saving treatments for patients with disease, if the diagnosis is not established and communicated promptly. Also, delayed result reporting can lead to affected infants being lost to follow-up and requiring repeat testing. Improving TAT helps ensure caretakers collect their results in a timely manner and minimizes the need for inefficient and expensive repeat testing. Ultimately, making improvements in all TAT phases for sickle cell screening is essential to optimize infant health outcomes, cost-effectiveness, and screening satisfaction for healthcare providers and patients in Uganda.

A major strength of this time analysis was the nationally representative long-term data from the CPHL centralized database, which allowed us to investigate previously undocumented parameters that affect sickle cell testing TAT in Uganda. Limitations include the access to data only for inbound TAT, which limits our ability to connect our findings to patient indicators such as notification and receipt of sickle cell results, followed by care and treatment of affected patients. Another limitation is the lack of recommendations for sickle cell testing TAT by the Uganda MOH, other regional programs, or by international bodies such as the WHO for a comparison of efficiency. However, our data provided the first detailed time analysis of sickle cell screening in sub-Saharan Africa, to enable monitoring and evaluation for future interventions and other programs.

Analysis of the direct costs of a sickle cell screening program in Uganda showed that the cost per test by IEF using dried blood spots was \$4.46. In Angola, the cost per infant screened in a newborn screening program that used IEF was \$15.36, or \$7.42 considering just the inputs of laboratory personnel, reagents, consumables, and equipment as in our study.<sup>142</sup> The cost of the screening test for sickle cell disease performed by IEF in the US was \$2.29 and approximately \$4.50 (reported as £3.51) in the UK.<sup>143,144</sup> The US and UK have addressed the cost-effectiveness of newborn screening for sickle cell disease for universal and targeted programs. Both nations have justified the greater cost incurred by screening every newborn for sickle cell disease, because it identifies all infants with disease, prevents more deaths, and has demonstrated better outcomes for patients.<sup>143-145</sup> In Africa, modelling simulations have found universal newborn sickle cell screening to be extremely cost-effective, especially in countries with a high disease prevalence of 0.2-0.5%.<sup>146,147</sup> Yet it remains that no federal newborn sickle cell screening currently exists in any country in Africa, despite the well evidenced economic and humanitarian basis for such programs.

In Uganda, the estimated costs per sickle cell disease case detected among the 10 regions varied from \$278.07 in East Central to nine times that in South Western (\$2607.19) (Table 6). These results show that newborn screening in regions with low sickle cell disease prevalence would result in a higher cost per case detected compared with screening focused in high-burden areas. However, because Uganda is a country with a high overall disease prevalence of 0.9%,<sup>123</sup> universal newborn screening would still be the most economical and impactful public health strategy for sickle cell disease in the country.

This analysis only considered costs and numbers of cases detected for a partial cost-effectiveness analysis. More extensive studies with patient follow-up data will be vital to provide evidence that screening is effective in reducing sickle cell morbidity and mortality, the benefits and harms of screening, and the long-term cost-effectiveness of a newborn sickle cell screening program in Uganda.

## Conclusion

This study provides a contemporary and detailed description of the time and costs of sickle cell screening in Uganda. Analysis of the different phases of TAT highlights areas for improvement to reduce the number of samples with excessive delays, and to strengthen the overall integrated CPHL sample continuum. The lack of sickle cell testing guidelines limits our ability to compare these results to screening and care standards, however, this study documents that the CPHL centralized database can accurately monitor and manage TAT for sickle cell screening and can identify factors affecting TAT at different phases to prompt targeted improvements. This study also shows that sickle cell testing by IEF is provided at under \$5.00, and the strategy of universal newborn screening is cost-effective to save and improve the lives of thousands of individuals with sickle cell disease in Uganda.

## **JOURNAL ARTICLE THREE**

### **Healthcare Providers' Experiences with Sickle Cell Screening in the Republic of Uganda**

#### **Introduction**

Sickle cell disease is group of genetic blood disorders that affects hemoglobin. Sickle cell anemia, the most common and severe form of sickle cell disease, is a monogenic disorder caused by a homozygous mutation in the beta-globin gene.<sup>1,2</sup> Individuals who inherit a single copy of the mutated sickle allele are called carriers of the sickle cell trait and are usually asymptomatic. The mutation leads to transformation of red blood cells into the characteristic crescent shape that causes chronic anemia, pain crisis, and serious infections.<sup>3</sup> These complications arise early in infancy, threatening the lives of affected babies if not immediately and adequately treated.

The epidemiology of sickle cell anemia has made it a global disease of public health importance. Sickle cell anemia is found all over the world and is the most common and rapidly growing genetic disease in sub-Saharan Africa.<sup>11,133</sup> Many of these countries have a sickle gene frequency of 10% to 40% resulting in more than 200 000 annual disease births and the associated under-five mortality is estimated to be as high as 50-90%.<sup>12,46</sup>

The primary step in the management of sickle cell anemia is early diagnosis through implementation of newborn screening followed by simple, but very effective prophylactic measures, such as penicillin prophylaxis and pneumococcal immunizations, the direct benefit of which is the considerable reduction of morbidity and mortality.<sup>134</sup> Sickle cell disease is an established component of national newborn screening programs in high-income low-prevalence countries, such as the United States and several countries in Europe.<sup>69,122</sup> These same interventions can be achieved in Africa, where national newborn screening does not currently exist, but where programs and improvements to basic healthcare are urgently needed to save the lives of many more affected children.<sup>44</sup>

Along with the logistical, political, and economic limitations in sickle cell endemic countries in sub-Saharan Africa, the lack of knowledge and awareness about the disease among healthcare providers and the community limits the potential for early and accurate diagnosis. In Uganda, sickle cell awareness among healthcare providers has increased as a result of the Uganda Sickle Surveillance Study (US3) and subsequent screening over the past five years.<sup>118,123</sup>

However, there is a paucity of information on their experiences with sickle cell screening since the service became newly available in the country in 2014. To address this gap, we undertook a qualitative research study to describe experiences with sickle cell screening among healthcare providers in Uganda.

## Methods

### *Sickle Cell Screening in Uganda*

Uganda's sickle cell screening program was established in 2014 as part of US3, the first national surveillance study of the burden of sickle cell trait and disease in sub-Saharan Africa. Following the one-year study, sickle cell screening continued with a strategic focus in districts considered to be high-burden. As previously described, sickle cell screening is based at the Central Public Health Laboratories (CPHL) as part of an integrated process of data collection, testing, and result reporting for various healthcare testing services.<sup>117,118,123</sup>

### *Study Setting*

The study was undertaken in Lira district in the Mid Northern region and Jinja district in the East Central region, and across three levels of health facilities. Over the past five years, from 2014 to 2019, Lira had 274 sickle cell disease positive results out of 1181 children screened (23.1%). Jinja screened a total of 2086 children, 637 of which were identified to have sickle cell disease (30.5%). Lira and Jinja were selected for this study because they are high-volume and high-burden districts achieved excellent coverage of sickle cell disease births at 92.6% and 87.1%, respectively. This is an indicator that healthcare provider suspicion is high and sickle cell screening utilization has been successful in these districts. The regional referral hospitals from each district were included in the study. Health center IIIs and IVs were chosen in each district based on the criteria of high-volume and high-burden.

## Recruitment Strategy

Purposeful sampling of healthcare providers from a broad range of disciplines and variable experience with sickle cell screening and management was done. Health facility administrators helped to identify key positions in the direct provision of sickle cell screening at their health facility. Using a snowballing recruitment technique, these healthcare providers were approached and asked to nominate other positions who could offer additional insights and perspectives on sickle cell screening.

Participants were approached at the health facility where they were provided with information about the study and asked to participate in an interview at a time that day that would be most convenient for them. At the time of the interview, participants were provided with informed consent and returned their signed forms before the start of the interview. Participants were assured of confidentiality and anonymity. Partaking in the study was voluntary, and participants were free to withdraw from the study at any time without prejudice. No participants who were approached refused an interview or withdrew their consent.

## Data Collection

Data were collected in October 2019 at individual interviews. A total of 23 interviews were conducted which lasted 20-45 minutes. Each interview was audio-recorded and supplemented with field notes taken by the study team.

A semi-structured interview guide was used in the interviews. The guide was designed to elicit information on how, when, where and by whom sickle cell screening services are provided at the health facility; the process of sample collection and result reporting to patients; sickle cell screening support and training received; perceptions of community knowledge and awareness of sickle cell disease; and perceived challenges of sickle cell screening and resolutions or proposals for improvement.

## Data Analysis

Interview audio-recordings were transcribed verbatim. Audio-recordings were reviewed against the transcripts by the study team. Field notes from individual interviews were also reviewed by the study team. Thematic analysis was done to identify emergent themes relating to the experiences of healthcare providers in screening for sickle cell disease. For reliability and validity of theme development, two members of the study team read and coded transcripts independently, then compared and discussed their coding to reach consensus around the final key themes.

## Results

### *Characteristics of Participants*

A total of 23 interviews were conducted with healthcare providers across the three health facility levels in two districts with representation from a variety of job titles (Table 1).

Five medical officers and 5 clinical officers participated in the study. A majority of the medical officers were recruited from the regional referral hospitals in both districts. Clinical officers were recruited from health center IIIs and IVs. Five laboratory personnel and 4 nursing officers from each level of health facility were interviewed. Three midwives and only 1 social worker were included in the study.

Table 1: Characteristics of the participants.

	Jinja District			Lira District			Total
	RR Hospital	HC IV	HC III	RR Hospital	HC IV	HC III	
<b>Medical Officer</b>	2	1	0	2	0	0	5
<b>Clinical Officer</b>	0	1	1	0	2	1	5
<b>Nursing Officer</b>	0	1	1	1	1	0	4
<b>Midwife</b>	1	0	1	0	0	1	3
<b>Laboratory</b>	1	1	1	1	0	1	5
<b>Social Worker</b>	1	0	0	0	0	0	1
<b>Total</b>	5	4	4	4	3	3	23

Abbreviations: RR Hospital, regional referral hospital; HC, Health center

## *Key Themes*

The key themes that emerged from the thematic analysis were training needs of healthcare providers on sickle cell screening, high volume of sickle cell patients and limited staffing, fragmentation of sickle cell screening and care, availability of screening supplies and necessary medications, and perceived community knowledge and attitudes towards sickle cell screening. Each theme is discussed in detail below:

### Training Needs of Healthcare Providers on Sickle Cell Screening

Across all healthcare provider job titles and health facility levels, the overwhelming response was that sickle cell screening training was nonexistent. Healthcare providers who play a clinical role in sickle cell screening, such as physicians, nurses, and midwives, were primarily responsible for identifying patients for screening. They discussed referring to their clinical education and on-the-job experience to recognize the signs and symptoms of sickle cell disease and decide how to screen their patient population. Within this group, there were four physicians and two midwives who could recall sickle cell disease as part of their educational curriculum to different extents. Those who did not receive any formal sickle cell training mentioned that they grew knowledgeable due to constant exposure to sickle cell disease patients, especially those who have spent a number of years in maternity and/or pediatric departments or units who have made sickle cell testing familiar in their practice and procedures.

*I never had training in the screening...but for us [physicians] by training we are taught. Like for us, to become a pediatrician whether you like it or not you have to go through a thorough training of sickle cell anemia, so we were trained by pediatrics hematologists. (Pediatrician, Regional Referral Hospital)*

*I was trained just for medicine; I did not train specifically [for sickle cell disease]. (Pediatrician, Regional Referral Hospital)*

*I have not yet had that training [on sickle cell disease] during my time for work but I had the training from when I was at school. (Midwife Health Center IV)*

*Since school I have not yet received any special training about sickle cell...I received the training when I was in school and I was attached to a sickle cell clinic at a regional referral hospital. (Physician, Health Center III)*

*Job training. For me, I never received any [sickle cell screening training] but job training. (Nurse, Regional Referral Hospital)*

The laboratory specific healthcare providers from assorted health facility levels included laboratory technician and technologist, as well as nurses and midwives, and were responsible for collecting and preparing sickle cell testing samples for transport. Almost all adapted what they learned from trainings established for other screening services, often citing the CPHL Early Infant Detection program.<sup>123</sup> There was one laboratory personnel who described a sickle cell screening training by the CPHL in 2015, but was the only one who attended from their health facility and has not encountered any similar training opportunities since. More commonly, the response was that there had been no sickle cell screening training opportunities provided by the CPHL.

There were few noted incidences of a lack of understanding on how to collect a sample, particularly from an infant and using a dried blood spot card with the added challenge of a busy setting, and how to indicate for sickle cell testing using the dispatch form.

*We have never gone for that [sickle cell screening] training...we are just taking off samples, but we have that knowledge gap and one time me myself I drew blood and they told me the results didn't come back because the blood was little. So, we have some knowledge gap and still it's existing even in screening. (Midwife HC III)*

The two regional referral hospitals that were visited had established sickle cell clinic days, which elevated the knowledge of sickle cell disease and interface with patients with disease to enable on-the-job training for healthcare providers even when formal sickle cell education was not previously provided. The provision of a sickle cell clinic also allowed for cross-training and cross-coverage of sickle cell care and screening activities, which was a major limitation for sickle cell services at lower health facilities.

*All people on board, all people, especially all health workers should have training, so that our index of suspicion goes up, because I may have the knowledge, but my colleague doesn't have enough knowledge and I don't sit here all days. (Physician HC IV)*

Overall, it was echoed by across all healthcare providers roles and facility levels that training in all aspects of sickle cell screening was greatly needed, from identifying patients for screening to sample collection to providing accurate information to caretakers during the relay of screening results. Among the few who were trained, several noted that single day and infrequent trainings at the facility were not sufficient for them to retain the information and master any new skills. The high rates of healthcare provider transfer and turnover was also discussed as a barrier to sickle cell screening that could be overcome with initial and ongoing training.

*I think it's needed very, very much for our facility here at least...because as much as we are doing the work, but we lack knowledge and experience...we read from books but we still need to learn more and more about it in order to help the people who are suffer with it. (Midwife HC III).*

## High Volume of Sickle Cell Patients and Limited Staffing

Across all health facility levels, human resources were extremely constrained. It was especially the experience for the regional referral hospitals, where the volume of the sickle cell patient population was immensely overwhelming for healthcare providers. The healthcare system in Uganda works on a referral basis, therefore the regional referral hospitals have become the catchment for sickle cell patients presenting with symptoms from the wide clinical spectrum of the disease, from basic care needs such as pain management to emergency crises including blood transfusion or even stroke.

*The other challenge we have is lower health centers are not working very well. Most of the regional referral hospitals are doing work of health center IIIs, health center IV and district hospitals across the whole Uganda. There are so many cases regional referral hospitals are treating and actually those babies would be better managed at Health Center IIIs, Health Center IVs and district hospitals. (Pediatrician RR Hospital)*

*Train the health center IVs...then it will lessen our work, we shall not have the numbers we always have here... they will be going there and they find the staffs are well versed like the way we're doing here then they will not end up coming here, first of all transport is expensive. (Pediatrician Physician Region Referral Hospital)*

Several healthcare providers at lower health facilities mentioned that they immediately refer patients with a positive sickle cell disease result to the regional referral hospital for all future care knowing they did not have the resources of knowledgeable staff and often availability of

basic medicines to be able to manage patients with sickle cell disease. They reported feeling that they do not have adequate backgrounds on sickle cell disease to be able to identify and provide proper management due to their lack of training and experience treating these patients, but many expressed willingness for these opportunities to be able to provide access to screening and care.

*Sickle cell management at health center levels is a challenge...I wish if we would be trained in that...specifically me I don't know which treatment specially we are supposed to give...and even what are the clinical features that we are supposed to look at in order to screen that blood for sickle cell. (Midwife HC III)*

#### Fragmentation of Sickle Cell Screening and Care

The process of sickle cell screening was different at each health facility, including the roles different healthcare providers performed in the process, where screening occurred, and timelines. The interviews revealed discrepancies within health facilities when healthcare providers in different job functions were asked to describe the flow of screening. This was most common at the high health facility level that had multiple entry points for sickle cell patients. For example, a regional referral hospital described two different areas where sample collection for sickle cell screening occurred, at the laboratory performed by a technician or technologist and at the vaccination point by a nurse or midwife. The biggest interruption in the sickle cell screening process that was stressed among all healthcare providers and facilities was the long turnaround time from sample collection to receiving the result from the CPHL. The reported turnaround time was between two weeks to more than a month, which was discussed to be the biggest cause of delay in care and patients being lost to follow-up. It was mentioned that in the

interim until result reporting, suspected disease patients would be seen multiple times in the hospital before receiving confirmation and appropriate care.

Once results are received, further issues were discussed regarding the fragmented care for sickle cell patients. Due to the many different disease complications that arise, sickle cell patients are often scattered among the different departments or units within a health facility, including maternity, pediatrics, out-patient, emergency, vaccination, and transfusion. The lack of a gravity point for patients is a significant barrier to sickle cell screening, especially when it comes to identifying patients for screening, streamlining screening flow, result reporting, and maintaining a medical history for patients. The two regional referral hospitals did have designated sickle cell clinic days that allowed for monthly follow-up, but specialized care outside of these days could not be guaranteed.

#### Availability of Screening Supplies and Necessary Medications

Screening supplies, including dried blood spot kits, are provided to all health facilities across the country by the CPHL. Samples are collected on dried blood spot cards for various testing services and are transported via the national sample transport system.<sup>14</sup> The organization of this system supports other operational needs, such as supply delivery. At the lower health facilities, occurrences of stock out of screening supplies were recounted, but were quickly remedied by the CPHL.

*[Screening supplies] ...we do run out of those. Even last week we ran short of it but they brought for us when we call they brought for us we had just received last week...actually it's rare to run out completely because every time we see the numbers are reducing we call and the hub riders they bring for us. (Laboratory Technician HCIV)*

Healthcare providers at all health facilities were greatly frustrated with the regular occurrence of stock out of basic medications and immunizations that are part of the routine schedule for all babies and young children. These treatments are essential for sickle cell disease patients, including penicillin and antimalarial prophylaxis, and the pneumococcal conjugate vaccine. Many of the healthcare providers identified weaknesses in the supply chain with supplies being inadequate or lacking completely.

*Actually most of the time drugs are not there especially pen v...so you will find them moving without drugs they are supposed to take...if their drugs could be easily available all the time...it makes it easier for them for their conditions... to begin treatment straight away when the drugs are even there. (OPD Nurse Regional Referral Hospital)*

Blood transfusions are a common treatment for sickle cell patients used to alleviate anemia and acute vaso-occlusive complications but are only available at regional referral hospitals who house blood banks for their regional catchment. However, blood supply shortages were noted as a common occurrence and it was even suggested that this was due to the immense number of sickle cell patients attended to at these facilities and referred from across the region.

The awareness of other disease modifying therapies was brought up by healthcare providers, such as hydroxyurea, a once daily oral medication that helps improve the laboratory and clinical complication of sickle cell disease. Hydroxyurea is the recommended treatment for patients with disease in the United States and is listed on the World Health Organization's Model List of

Essential Medicines for Children<sup>148</sup> and adults.<sup>149</sup> Unfortunately, it is not yet widely available in Africa, but local clinical evidence and experience with its use for sickle cell disease is being generated by several ongoing clinical trials in Uganda and other African countries.<sup>108-112</sup>

*Number one we need a blood bank... secondly if the government would make provision of hydroxyurea... we would save so many people from the other complications and hospitalization... (Physician Regional Referral Hospital)*

*Hydroxyurea. It's been a wonder drug. If there is a way the Ministry of Health can adopt and adapt to what has been published to help sickle cell. Because they're very many studies but I think it has taken time to adopt some of these publications...one due to politics, two fear of funding. B I believe Ministry of Health can take this up and see how it can prioritize the budget to sickle cell. (Physician Regional Referral Hospital)*

#### Perceived Community Knowledge and Attitudes Towards Sickle Cell Screening

Healthcare providers discussed community knowledge and attitudes about sickle cell disease as a major challenge of screening. The lack of genetic knowledge about the disease and the invisibility of carrier status often leads to blame placed on mothers, including a risk of blame for misaligned paternity. The risk of accusations of unfaithfulness was mentioned in all interviews with many detailed accounts, and it was generally due to the fathers being unable or unwilling to accept a role in causing the disorder in their children.

*Some of them end up crying... because of emotional problems they're overwhelmed and whatever they cry, some of them cry because of the blames (Physician Regional Referral Hospital)*

*In the community, I think the perception of sickle cell itself is still so poor. They don't understand what sickle cell is for example most couple have a blame on one couple... especially the men blame the female. (Physician Regional Referral Hospital)*

*A couple of five children, the four were normal...but the fifth had sickle cell...and then when they brought...the father said this is not my child. I want this child to die. (Counselor Regional Referral Hospital)*

The lack of sickle cell knowledge in the community is replaced by significant misconceptions that lead to aberrant attitudes toward the disease, such as the thought that sickle cell disease is caused by a curse in the family or witchcraft. It was noted that this thinking was still common thinking among rural communities and villages who often practice alternative approaches to medicine, such as herbal remedies or spiritual solutions.

*They are bewitched they are bewitched...there is a curse in that family...they don't know the disease. People deep there in the village they don't know the disease. (Midwife HC III)*

It was overall reported that the insufficient knowledge of the nature of the disorder, negative attitudes, and stigmatization add to the burden imposed on patients and their families, greatly influencing health-seeking behaviors, such as screening and attending clinic.

*Parents tend to be reluctant, what normally brings that is the sickness. When the child is ok, they don't even come back...when you tell them to come back after a month...the greater percentage, they don't tend to come back. (Physician HC III)*

Many of the healthcare providers detailed the counselling they provided for sickle cell patients and caretakers to manage the knowledge gap. Their communication was focused on generating an understanding of the concept of the inheritance of the disease and, therefore, acceptance of the role of both parents to overcome the risk of blame and stigmatization of children with disease and mothers.

## Discussion

This qualitative study has explored the experiences of healthcare providers with sickle cell screening in Uganda. Essential insights from healthcare providers in the direct provision of sickle cell screening helped us to identify barriers to screening and propose resolutions, including the need for initial and ongoing training for healthcare providers, healthcare system capacity issues, such as resources concerning staffing and supplies, and system fragmentation; and the knowledge and awareness gap of sickle cell disease in the community.

Although there was adequate awareness of sickle cell disease among healthcare providers, this did not always translate to awareness of the sickle cell screening process or meaningful knowledge that is useful for screening, such as the detection of patients for testing. This was especially true at lower health facility levels of health centers III and IV that were limited in their knowledge of diagnosis and management of sickle cell disease. Initial and ongoing training of the sickle cell screening process and care will bridge training gaps due to high healthcare provider transfer and turnover rates. Through regular training, healthcare providers at lower health facility levels will become more aware of the disease and basic principles of disease management to help alleviate the high volumes of patients at higher level facilities due to mass referrals.

The regular stock out of basic medications, such as antibiotics, antimalarial drugs, and important childhood vaccinations put children with sickle cell disease at a high rate of death from dangerous infections. Procurement and disruption of essential medicines is revealed to be a major challenge at all health facility levels; therefore, procurement planning should be based on disease burden to ensure accuracy of planned quantities and informed by healthcare providers directly. Furthermore, beyond the standard treatments that will continue to be needed for the prevention of malaria and bacterial infections, the increasing prevalence of sickle cell disease in African countries will progressively burden healthcare systems and communities without the wide-spread availability of disease-modifying therapies. Blood transfusion is commonly used to alleviate anemia and pain crisis caused by sickle cell disease, though the regular blood shortages pose a serious risk for patients. Hydroxyurea treatment has been shown to be safe, efficacious, and feasible in Uganda, however, the implications are limited by the availability of the drug, the costs of treatment, and laboratory monitoring that is needed.<sup>108-112</sup>

Sickle cell disease is a multisystem condition that has a wide range of complications, which makes sickle cell patients vulnerable to the fragmentation within health facilities leading to decreased service delivery and care quality. The best practice approach would be the establishment of sickle cell departments or units at higher health facility levels that could provide the support of designated clinic days at lower health facilities. This care model for sickle cell disease would streamline screening, and orient staffing and supply needs for comprehensive sickle cell care.

Healthcare providers perceived that the community had inadequate knowledge and negative attitudes towards sickle cell disease that impacted health-seeking behavior, such as screening. A resolution that was given was a widespread community intervention strategy, such as mass communication via television, radio, or other media programs backed by the Ministry of Health to provide accurate information about sickle cell disease. The deployment of community health workers and faith leaders as advocates would further promote the messages and have a farther reach to rural parts of the country.

### *Strengths and Limitations*

A strength of this study was the qualitative study design, because the open-ended data collection method is best suited to provide real-life context and in-depth understanding of healthcare providers' experiences with implementation, procedures, practices, perceptions, and challenges of sickle cell screening. Another strength of this study was the systematic approach used in sampling, data collection and analysis to enhance reliability and validity of the analysis: checking of transcripts against audio-recordings and field notes taken. In addition, purposeful sampling was used to select a wide range of providers with different experiences. However, limitation of this study was that the sample was narrowed to two high-burden districts, Lira and Jinja, therefore, these findings may not be generalizable to all districts in Uganda.

## Conclusion

This is the first study to describe the challenges and realities of sickle cell screening experienced by healthcare providers in Uganda. Our results will inform targeted strategies for strengthening and build-up of the country's current sickle cell screening program through healthcare provider training, community education, and improved sickle cell care models, with the ultimate goal of universal newborn sickle cell screening in Uganda.

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## APPENDICES

### Appendix A: Sickle Cell Healthcare Provider Experience Semi-structured Interview Guide

<b>Sickle Cell Healthcare Provider Experience: Sickle Cell Clinic Personnel</b>
<p><b>AFTER THE INTERVIEW, THIS PAGE WILL BE DETACHED FROM THE DOCUMENT AND FILED SEPARATE FROM THE INTERVIEW NOTES AND RECORDINGS.</b></p> <p><b>DO NOT WRITE ANY OF THE PARTICIPANTS’S IDENTIFYING INFORMATION ON THE INTERVIEW NOTES ON PAGES 2 AND 3.</b></p>
Date of Interview (date/month/year):
District:
Health Facility:
Health Facility Level: <ul style="list-style-type: none"> <li><input type="checkbox"/> Regional Referral Hospital</li> <li><input type="checkbox"/> District Hospital</li> <li><input type="checkbox"/> HC IV</li> <li><input type="checkbox"/> HC III</li> <li><input type="checkbox"/> HC II</li> </ul>
Name of Person being Interviewed:
Job Title of Person being Interviewed: <ul style="list-style-type: none"> <li><input type="checkbox"/> Medical Officer</li> <li><input type="checkbox"/> Clinical Officer</li> <li><input type="checkbox"/> Nursing Officer</li> <li><input type="checkbox"/> Nurse</li> <li><input type="checkbox"/> Other: _____</li> </ul>
Gender of Person Being Interviewed: <ul style="list-style-type: none"> <li><input type="checkbox"/> Male</li> <li><input type="checkbox"/> Female</li> </ul>
Telephone and/or Email Contact of Person being Interviewed:

**Sickle Cell Healthcare Provider Experience: Sickle Cell Clinic Personnel  
Semi-Structured Interview Guide**

Recording Start Time (using 24 hours):

1. Tell me about your work. How did you end up working in the sickle cell clinic?
2. Your clinic has been offering newborn sickle cell screening and I would like to know your experience implementing it. Tell me how it started and how you implemented it.
3. What specific role do you have in newborn sickle cell screening?
4. Tell me about the process from collecting the sample from the patient to when the caretaker receives result.
  - *Probe: How do you select who to screen?*
  - *Probe: What happens after the result is given to the caretaker?*
5. How has the Ministry of Health/Central Public Health Laboratories supported you in implementing newborn sickle cell screening?
6. Tell me about the training you have received on newborn sickle cell screening.
  - *Probe: What was helpful about the training?*
  - *Probe: What additional training is needed?*
7. Who in your clinic has received training on newborn sickle cell screening?
8. What are some challenges you have experienced implementing newborn sickle cell screening?
  - *Probe: How have you resolved these challenges or how do you propose they be resolved?*
9. Tell me about the range of issues you have experienced with other healthcare providers and caretakers when it comes to newborn sickle cell screening.
  - *Probe: What kinds of issues with acceptability of newborn sickle cell screening have you experienced with other healthcare providers and caretakers?*
  - *Probe: Tell me about any ethical scenarios that have risen? For example, rejection of the test result or paternity concerns?*
  - *Probe: How have you resolved these issues or how do you propose they be resolved?*
10. If you were to advise another clinic that would like to implement newborn sickle cell screening, what would you tell them to do that your clinic has done well? What would you tell them to do differently from what you have done?
11. If you were to advise the Ministry of Health/ Central Public Health Laboratories about scale-up of the newborn sickle cell screening to other health facilities, what would you tell them to do that they have done well? What would you tell them to do differently from what they have done so far?

12. Is there anything else that you would like to tell me about newborn sickle cell screening at this health facility?

Recording Stop Time (using 24 hours):

*"Thank you so much for your time."*

## Appendix B: Sickle Cell Healthcare Provider Experience Semi-structured Interview Guide Consent Form

UGANDA MINISTRY OF HEALTH/MAKERERE UNIVERSITY/CINCINNATI CHILDREN'S HOSPITAL

Project Title: Prevalence and Mapping of Sickle Cell Trait and Disease in Uganda

Objective: Healthcare providers' experiences with sickle cell screening in Uganda

### **INFORMED CONSENT TO TAKE PART IN RESEARCH**

#### **Introduction**

We are inviting you to be in a research study conducted by a study team comprised of members from the Uganda Ministry of Health, Makerere University College of Health Sciences, and Cincinnati Children's Hospital Medical Center (USA). The purpose of the study is to describe healthcare providers' experiences with newborn sickle cell screening. The goal is to identify any opportunities for improvement, with the overarching goal of strengthening the current healthcare system to provide optimal sickle cell diagnosis and care for patients in Uganda.

#### **Procedures**

You have been selected to participate in this interview because of the important role you play in newborn sickle cell screening services and providing care to children with sickle cell disease. If you agree to participate in this study, you will first provide informed consent complete with your signature and the date. You do not have to be in the study if you do not want to; it is your choice and you can change your mind at any time.

You will be interviewed by a member of the study team. The interview will be conducted in English and will take about 30 to 60 minutes of your time. If you do not wish to answer any question during the course of the interview, please let us know and we will move to the next question. To capture your responses during the interview, an audio-recording will be taken. Personal identifiers, such as your name and the name of the health facility, will not be included in the audio-recording.

#### **Confidentiality**

All information collected about you will be kept private and treated with strict confidentiality. Your name will not be used in any research reports or publications that may arise from this study.

The audio-recording and transcription of the recording will not contain any personal identifiers and will be kept on a password protected computer and in a locked file cabinet when not in use that is only accessible by select members of the study team. The audio-recording and transcription of the recording from this interview will be destroyed when the study is completed.

## **Benefits**

There is no immediate direct benefit to you as a result of participating in this study. However, results from this study will be available to policymakers, implementing partners, and donors and will inform interventions to improve newborn sickle cell screening services for patients in Uganda.

## **Risks/Discomforts**

There is no major risk associated with participating in this study. Some of your time will be spent while you complete the informed consent and for the interview. The interview will be scheduled at a time when it is most convenient for you and in a place that is most comfortable for you. During the interview, please feel free to seek clarification regarding the interview questions and to not answer questions you find difficult or uncomfortable to respond to. You may skip any question or stop the interview at any time.

We will make every effort to keep your information confidential; however there is always a small risk of unwanted or accidental disclosure.

## **Study Withdrawal**

Your participation in this research study is entirely voluntary. You can choose to participate or not. Participating or not will not affect your job or work evaluation in any way. You may also stop participating in this study at any time with no consequences and all information collected about and from you will be discarded.

## **Cost and Compensation**

If you decide to take part in this research study, you will not incur any costs. You will not be provided money or any other type of incentive for participating in this study.

## **Questions**

This research study has been approved by the committees that are responsible for protecting people participating in research in Uganda.

If you have questions about the study, please contact the study lead: Arielle G. Hernandez MPH on +12818816845 and/or [arielle.hernandez@cchmc.org](mailto:arielle.hernandez@cchmc.org).

If you have any complaints, suggestions, or questions about your rights as a research volunteer, please contact the Chairperson of the Makerere University School of Medicine Research and Ethics Committee (SOMREC): Prof. Ponsiano Ocama on +256772421190 and/or [ponsiano.ocama@gmail.com](mailto:ponsiano.ocama@gmail.com).

## Signatures

Please sign below if you choose to participate in the study and fully understand that your participation is voluntary and you can withdraw at will without any consequences. Furthermore, please sign below if all of your questions have been sufficiently answered and you fully understand the purpose of the study and all the information given to you regarding the procedures, benefits/risks, and confidentiality.

_____ Printed Name of Subject	_____ Signature of Subject	_____ Date
_____ Printed Name of Person Obtaining Informed Consent	_____ Signature of Person Obtaining Informed Consent	_____ Date

REC #: 2012-138  
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