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The Pressing Need for Point-of-Care Diagnostics for Sickle Cell Disease: A Review of Current and Future Technologies

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Abstract

Sickle cell disease (SCD) is a common and life threatening inherited blood disorder, affecting over 300,000 newborns per year. Over 75% of SCD births occur in sub-Saharan Africa, where the lack of timely and accurate diagnosis results in premature death within the first few years of life for a majority of affected infants. Current methods to diagnosis SCD require expensive laboratory equipment and reagents, and adequately trained laboratory personnel. In addition, test results are often delayed due to transport and batching of samples in a central laboratory. Financial and technical limitations often preclude any form of SCD laboratory testing at the local level in regions where SCD is most prevalent. There has been a recent surge of interest in addressing the global burden of SCD, including improving and optimizing diagnostic capacities. Largely stimulated by a funding opportunity from the NIH, several point-of-care diagnostics have been developed for SCD with a focus on developing devices that are inexpensive, simple, and practical in limited resource settings. In this manuscript, we review the global burden of SCA, including the rationale for the development of POC assays, and carefully review the POC devices currently in development.

Keywords

sickle cell disease; global health; Africa; diagnostics; point-of-care

INTRODUCTION

The hemoglobinopathies, including both sickle cell disease (SCD) and the thalassemias, comprise a heterogeneous group of inherited red blood cell disorders caused by mutations in the globin genes. Due to the heterozygote advantage against malaria, approximately 7% of the world's population are carriers of a significant hemoglobin variant.[1] Globally, over 300,000 infants are born with the most severe form of SCD (homozygous HbSS disease) with a majority of these births occurring in sub-Saharan Africa, where mortality before five years of age is estimated to be as high as 50–80%.[2, 3]

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In the well-resourced settings of the USA and most of Europe as well as the middle-resourced setting of Jamaica, early identification of SCD through newborn screening (NBS) is routine and subsequent provision of preventive measures and comprehensive care is highly effective in reducing morbidity and early mortality.[4–8] In these countries, NBS for hemoglobin disorders involves coordinated systems for laboratory testing, reporting and follow-up. NBS for hemoglobin disorders is often performed in conjunction with testing for other inherited disorders using a portion of the dried blood specimen collected at birth. In these settings, diagnostic testing is structured around a centralized reference laboratory and, although methods may vary between programs, the workflow for NBS is essentially the same: specimen collection and transport, laboratory testing and reporting, and follow-up to verify screening results and confirm the diagnosis. Outside of the USA and Europe, particularly in regions where the incidence of SCD can be as high as 1–2%, NBS is limited to only a few pilot programs and no country has yet adopted universal NBS. [9–14] Recent data from the few sub-Saharan African countries where pilot NBS programs for SCD have been initiated (Benin, Nigeria, Ghana, Congo Democratic Republic and Angola) indicate that preventive measures have reduced infant and under-5 year mortality in affected newborns successfully located and brought to care.[13–16] Despite the proven efficacy and relatively low cost of NBS, large-scale implementation remains impractical in many sub-Saharan countries in part due to inadequate financial, laboratory, and technical resources to carry out currently available SCD diagnostics. The development of low-cost, rapid, and easy to use diagnostics for SCD may circumvent these barriers and make expansion of NBS in regions of Africa with limited infrastructure and resources a feasible goal.

Traditional Diagnostic Methods

There are a number of highly accurate and validated diagnostic methods for SCD, including cellulose acetate and citrate agar electrophoresis, isoelectric focusing (IEF), high-performance liquid chromatography (HPLC), capillary zone electrophoresis and DNA analysis. These laboratory methods are briefly reviewed here with a focus on the relative complexity and cost of each.

Classical Electrophoresis: Cellulose acetate and Citrate Agar—Hemoglobin electrophoresis is the traditional method used for the separation and identification of normal and variant hemoglobins in blood applied to a filter paper matrix.[17] The process is based on the differential mobility of charged molecules across a support medium in an electric field. Hemoglobins are separated based on differences in charge and identified by specific banding patterns. Co-migrating hemoglobins may be further separated by varying the support medium or the pH of the electrophoretic solution and a two-tiered electrophoresis protocol at alkaline and acidic pH is typically employed and begins with cellulose acetate electrophoresis (pH~8.6) followed by citrate agar (pH~6.0). Visual estimation of band intensities and scanning densitometry may be used to distinguish sickle cell trait (Hb AS) from Hb S/ β^+ thalassemia (Hb SA). Given the relatively low cost of equipment, reagents and supplies, electrophoresis is likely the least expensive method for hemoglobinopathy diagnosis, but is time- and labor-intensive.[18]

Isoelectric Focusing (IEF)—IEF is another electrophoretic technique used to separate hemoglobins based on their net charge within a pH gradient on a gel medium. IEF is an equilibrium process in which hemoglobins migrate within a pH gradient to a position of zero net charge, or isoelectric point (pI). The gradient is set to optimize resolution within a narrow range of pH 6.8 to 7.8 where most hemoglobin variants reach their pI. Unlike classical electrophoresis, which often requires both basic (cellulose acetate) and acidic (citrate agar) pH assays to resolve hemoglobins with a similar migration pattern, most hemoglobins have a distinct pI and can be differentiated by IEF. The narrow bands obtained on IEF require less expertise to interpret and allow for more precise and accurate quantification than standard electrophoresis. As with classical electrophoresis, IEF is labor-intensive and time-consuming and provides only semi-quantitative results.[19]

High-performance liquid chromatography (HPLC)—Cation-exchange HPLC is a method that is also based on the net charge of the hemoglobin molecule at a specific pH, but is fully automated and provides precise quantification of hemoglobins.[20] Hemoglobins adsorbed to a negatively charged resin column (stationary phase) are eluted from the column by a positively charged solution that is added in increasing concentration and competes for binding to the negatively charged resin. Hemoglobins are thus differentially eluted at a rate related to their affinity for the column. The resulting elution patterns (Hb peaks) are graphically represented in defined windows based on their relative time to appearance in the eluate (retention time). The size of the Hb fractions can be quantified by computing the area under the curve for each peak. Commercial cation exchange HPLC systems have been adapted for high throughput NBS for hemoglobinopathies by shortening the retention times. Major advantages of HPLC are automation with rapid throughput on average 1500 samples per day with a sample run time of 2 minutes. Automated systems for NBS contain computer software to identify various hemoglobins by comparison of retention times to a reference library and integrate peak areas to quantify the relative amounts of hemoglobins detected. Limitations include cost, technical expertise, high sensitivity to degraded samples, and the requirement for regular and careful instrument calibration and maintenance by experienced personnel.

Capillary Zone Electrophoresis (CZE)—CZE is a hybrid technique combining classical electrophoresis with liquid chromatography. A liquid flow electrophoresis in which buffer has replaced the solid support medium (e.g., agarose gel) separates Hb based on the pH of the buffer. Hemoglobins are separated in an electrical field using alkaline buffers in small silica glass tubes (capillaries). Hemoglobins migrate toward the cathode in the capillary tube by electro-osmotic flow and optical density detected by ultraviolet (UV) system. The migration time of the hemoglobin variant (from injection of the sample to detection of a specific molecule) is divided into fifteen (15) zones. Like HPLC, sampling and data acquisition are automated. Commercial systems incorporate 8 capillaries in parallel to allow 8 samples to be tested simultaneously. The most common variants (e.g., Hb S, C, E, and D) are identified by their zone with a specificity >90%. However, as with other protein-based methods, there is overlap of hemoglobin variants in a particular zone. As with HPLC, there is significant cost associated with the equipment and reagents, a high degree of laboratory expertise is required for sample processing and interpretation.

IEF, CZE and HPLC are currently considered the gold standard methods for diagnosis of SCD. These methods are well suited to screening programs designed around specialized facilities with timely collection and storage of samples, stable electrical power, refrigeration, automated instrumentation and trained personnel to perform testing. However, each method has limitations when considering potential widespread application in settings with low resource settings, including sub-Saharan Africa.

Table 1 summarizes currently available techniques for Hb detection, including estimated cost of equipment and reagents, sample processing time, and specific limitations/advantages. In addition to the specific technical limitations of these methods, the time required for sample transport and processing increases the time to reporting of results by several days to weeks. In limited resource settings, it is often difficult or impossible to relay positive results to patients due to disconnected or inaccurate cellular phone numbers and the lack of specific numerical street addresses. Simple and inexpensive SCD diagnostics designed for use at the point-of-care (POC) to provide results within minutes at the time of a clinical encounter, vaccination visit, or prior to discharge of infant and mother after delivery would fill a tremendous unmet need for SCD diagnostic capacity in limited resource settings.

Recognizing the Need for Point-of-Care SCD Diagnostics

The burden of SCD is becoming increasingly recognized on a global scale by large organizations including the United Nations,[21] World Health Organization (WHO),[22] the National Institutes of Health (NIH),[23] and the American Society of Hematology (ASH), with an identified urgent need for low-cost and accurate POC diagnostic devices for SCD in high prevalence, low resource areas. With increased recognition and associated funding opportunities, there has been an upsurge in development of POC assays for SCD in the past several years. In particular, the National Heart, Lung, and Blood Institute has utilized the Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs to encourage the development and implementation of new POC technologies. These SBIR/STTR programs allow US-owned and operated small businesses to engage in federal research and development that has a strong potential for commercialization.[24] Several small businesses have utilized the SBIR/STTR mechanism to promote the development of a POC device for the diagnosis of SCD in low resource settings. The funding opportunity for the development of SCD POC assays included phase 1 development and feasibility testing, followed by phase 2 testing of the device in targeted populations in low resource setting. A framework for evaluating POC tests has also been proposed to determine a diagnostic test's capacity to support a clinical decision within its operational context ("test efficacy"), as well as revised criteria for an ideal diagnostic POC test in resource-limited settings.

Criteria for an acceptable POC test in limited resource settings—For optimal use in low-resource settings, it is important for tests to be rapid (fast turnaround to provide treatment and education at same visit), simple to use (requiring few facilities, equipment or training), low-cost, easily interpretable, and stable when transported and stored under extreme temperature and humidity conditions. The WHO initially developed the 'ASSURED' criteria for an "ideal" POC test in resource limited settings (as part of the

Sexually Transmitted Disease Diagnostics Initiative).[25] The ASSURED criteria are defined below with specific comments about applicability to SCD testing.

- **Affordable** by those at risk – end user cost for SCD POC tests has not yet been determined, but less than \$5 per test or ideally less than \$1 would allow for widescale implementation
- **Sensitive** (few false-negatives) – detection of HbS in newborns with high HbF
- **Specific** (few false-positives) – distinct detection of HbS, HbC, HbA – ability to distinguish trait (HbAS) from disease (HbSS, HbSC, HbS/β-thalassemia)
- **User-friendly** (simple to perform, easy and intuitive interpretation, and requiring minimal training)
- **Rapid** (results available during visit)/**Robust** (device has a long shelf-life, does not require refrigerated storage)
- **Equipment-free** (no additional equipment, e.g. refrigeration, electricity required; ideal device is compact, portable, low weight)
- **Delivered** to those who need it –available in rural and urban communities in areas of high prevalence

In addition to laboratory-based assessment of test sensitivity and specificity, clinical studies are required to address the utility, accuracy and cost-effectiveness of new devices in the hands of the end users at the point of care in limited resource settings.

Innovative Point-of-Care Technologies for SCD

Lateral Flow Immunoassays—Lateral flow immunoassays (LFIA) are among the few low-cost, rapid, ready to use diagnostic tests to be successfully applied in low resource settings.[26] As no special instruments or environmental conditions are required and results are available within minutes, LFIAs are ideal for POC diagnoses in areas without access to a specialized clinical laboratory. Most LFIA devices utilize high quality antibodies that result in excellent (>90%) sensitivity and specificity. The commercial availability of antibodies with high specificity for several normal and variant hemoglobins make LFIA an excellent choice for POC diagnosis of SCD. Once developed, LFIA are amenable to large-scale, affordable production using relatively inexpensive, off-the-shelf components and reagents. Often referred to as a “dipstick” assay, LFIA can be formatted for detection of either antigens or antibodies in various clinical specimens. Most are stable at ambient temperatures for more than a year when appropriately packaged and can be shipped and stored without refrigeration. Given the widespread use and universal familiarity with rapid malaria and HIV tests using similar lateral flow platforms, an LFIA SCD test is the method most aligned for success in the healthcare settings in Africa.

Formats for LFIA include the standard “sandwich format” and “competitive format”.

In sandwich format LFIA, target antigens bind to labeled (capture) antibodies and are detected by binding to immunospecific (detection) antibodies immobilized on a membrane

strip at the test line. In the competitive format LFIA, labeled antigens are dried on polypropylene surface in a test vial.

Monoclonal (capture) antibodies and the control capture antibody are fixed to test lines on nitrocellulose membrane strip.

Sickle SCAN®—The Sickle SCAN® assay (Biomedomics, Inc., Research Triangle Park, NC, USA) is a sandwich format chromatographic immunoassay using polyclonal antibodies against human HbS, HbC, and HbA for the qualitative assessment of each of these hemoglobins in order to distinguish normal, sickle cell trait (HbAS) and the two most common forms of SCD (HbSS, HbSC).[27, 28] The three polyclonal antibodies against human sickle hemoglobin (HbS), hemoglobin C (HbC), and normal adult hemoglobin (HbA) are the capture antibodies which are fixed on the strip (test lines). A separate capture antibody is fixed on the control line. A labeled monoclonal antibody against the α -globin chain present in all hemoglobins is used as the detection antibody to provide the visual read-out. The Sickle SCAN kit includes tubes prefilled with 1.0mL of buffer which lyses erythrocytes and releases hemoglobin. The hemolysate is prepared by mixing 5 μ L of capillary blood (most easily collected by finger stick) with the buffer; after mixing, five drops of the hemolysate are then applied to the test cartridge. DBS samples may also be tested by dropping a 3mm punch into the prefilled buffer container, mixing and applying 5 drops to the test cartridge. As the hemolysate test sample diffuses through the absorbent test strip, the labeled detection antibodies bind the hemoglobins present in the sample, forming antigen-antibody complexes. The antigen-antibody complexes selectively bind to the corresponding capture antibodies on the test lines, producing a blue colored band for each type of Hb present. Figure 1 illustrates the read out for common hemoglobin genotypes.

Laboratory testing of this assay on whole blood samples from 137 heterozygous and homozygous individuals accurately detected Hb A, Hb S, and Hb C with 99% sensitivity and 99% specificity. In this initial study, the results were interpreted using customized software to analyze scanned images of the test bands. The LFIA was then tested at the POC using capillary samples collected at the bedside from another 71 subjects with known Hb A, S, and C genotypes. The test results were compared to HPLC and/or capillary zone electrophoresis (CZE) and showed 100% sensitivity and near-perfect specificity.[27]

Further validation of the the Sickle SCAN device was performed using a visual readout in a separate analysis of 139 venous blood samples from individuals, including newborns, with normal and variant hemoglobins. Compared to CZE, the device had a high sensitivity (98.3–99.5%) and specificity (92.5–94%) for detection of HbS and HbA, and 98.4% sensitivity and 98.6% specificity for distinguishing SCD from SCT and normal blood.[28] The device yielded similar results using dried blood spot samples. Devices stored at 37°C and blood samples stored at 4°C for up to one month gave identical results to those stored at room temperature. Importantly and in contrast to many other newly developed POC tests, Sickle SCAN is able to clearly distinguish HbAA, HbAS, HbSS, and HbSC without interference from even high levels of HbF. This allows for use of this device as an accurate diagnostic for newborns with high levels of HbF.

Collectively these results indicate that the Sickie SCAN test meets several criteria for an ideal POC device. With rapid, accurate, sensitive and specific results obtained from both liquid blood and dried blood spots, including those from newborns with elevated HbF levels, this device seems suitable for large-scale SCD screening in limited resource settings. Limitations of the Sickie SCAN device include the fact that it is a qualitative test, which could result in a test interpreted as sickle cell trait (HbAS) in individuals with HbS/ β + thalassemia. While the limit of detection (LoD) for Hb S and Hb C is 1% and 2%, respectively, the assay's LoD for Hb A was reportedly pre-defined at 40% to ensure distinction of sickle cell trait (Hb AS) from SCD.[27] Despite this pre-set LoD, a clear band for HbA is reportedly visible with HbA concentrations as low as 5–10%. [28] Samples containing Hb S and Hb A as low as 5–10% will show a positive band for Hb S and Hb A and be interpreted as sickle cell trait, whereas samples with Hb A < 5–10% may show only a single positive band for Hb S and maybe falsely interpreted as SCD. Thus, the test provides a qualitative read-out only, indicating the presence but not the quantity of HbA, HbS, or HbC. The variable intensity of the test bands is another limitation that may lead to potential misinterpretation of results. For example, tests from persons with sickle cell trait (HbAS) in whom Hb A levels are always greater than Hb S levels will visibly demonstrate a stronger HbS band than HbA band. The naïve user may misread the presence of an S band that is much stronger than an A band as SCD. Another minor limitation of this device is the potential interference with Hb A by cross-reacting Hb variants. Because the Sickie SCAN prototype uses polyclonal antibodies to detect each hemoglobin type, variants such as Hb D-Punjab (Los Angeles), Hb E, Hb O-Arab which share the same epitope as Hb A (cross-reacting Ab) may be falsely read out as Hb A. Finally, the accuracy and clinical impact of this POC test still needs to be proven in individuals with SCD in low resource countries for which it is intended. Although developed for low-cost testing in resource poor settings such as sub-Saharan Africa and India, the specific cost to users still needs to be determined.

The published results come from studies performed using a prototype Sickie SCAN device and the final marketable product has yet to be established. The test could be improved by increasing the affinity or concentration of HbA antibodies or by increasing specificity by employing monoclonal instead of polyclonal antibodies. This change would be especially relevant to newborn for screening in which detection of a small (<10%) amount of HbA can make the difference in interpretation between disease and carrier states. Finally, the Sickie SCAN assay requires a very small amount of blood collected by fingerprick, but the user must perform an extra step diluting the blood in the supplied buffer solution before adding it to the device. Direct application of the finger stick blood sample, as is common in most LFIAs, could simplify the test operation and reduce cost.

HemoTypeSC™—The key component of a successful LFIA for SCD diagnosis is the specificity of the antibodies used to detect normal and variant hemoglobins. In contrast to the Sickie SCAN LFIA which uses polyclonal antibodies for detection of Hb A, S, and C, the HemoTypeSC™ assay (Silver Lake Research Corporation, Azusa, CA, USA) incorporates monoclonal antibodies (MAbs) with greater specificity for Hb A, S, and C and ability to resolve and distinguish normal from common carrier and SCD genotypes.[29] Prior to the development of the competitive LFIA, in vitro screening by direct and

competitive ELISA assays was performed to select the MAbs with highest affinity and specificity for Hb A, S, and C. Monoclonal antibodies with <0.1% cross-reactivity with non-targeted Hb variants were selected for use as capture antibodies for the LFIA POC device. In this competitive lateral flow assay format, labeled reagent antigen competes with sample antigen to bind to the immobilized MAb on the membrane. Hemolysate is prepared by adding 1 μ L whole blood to 1mL of H₂O and applied to the test strip which is placed in the vial containing buffer. After 10 minutes, the test strip is removed from the vial and visually inspected for the presence or absence of a red-colored band at each of four test lines (Hb C, Hb S, Hb A and control). Each type of hemoglobin present in the sample competes with labeled HbA, HbS and HbC antigens for binding to the corresponding immobilized capture MAbs on the test strip. Presence of a Hb variant in the sample is indicated by the absence of a red band at the test line. Figure 2 illustrates the design and interpretation of the HemoTypeSC device.

The HemoTypeSC assay was clinically validated in the laboratory using whole blood samples from 100 individuals with known Hb phenotypes including normal (Hb AA, $N=20$), carrier or trait (Hb AS, $N=22$; Hb AC, $N=20$), SCD (Hb SS, $N=22$; Hb SC, $N=13$), and Hb C disease (Hb CC, $N=3$) as previously diagnosed by CZE or HPLC and confirmed by cellulose acetate, citrate agar electrophoresis or IEF. The correct hemoglobin phenotype was identified in 100% of samples. Test accuracy was not affected by the amount of Hb F (0–94.8% of total Hb) or Hb A₂ (0–5.6% of total Hb).[29]

This LFIA overcomes the specificity and sensitivity limitations of the Sickie SCAN device through the use of highly specific monoclonal Abs rather than polyclonal antibodies to distinguish hemoglobins. The assay does not require a separate dilution step for detection of antigens present at high concentration. HemoTypeSC may be a practical solution for point-of-care testing for SCD and carrier status in low-resource settings. The device is low cost (less than \$2), demonstrates high sensitivity and specificity, simple (requires <1 μ L of blood by fingerprick, without instruments or a power source), user-friendly (simple to perform with minimal training), rapid (total time-to-result of <20 min), robust (does not require refrigerated storage), and equipment-free (e.g no refrigeration, electricity required). A potential limitation when used in the field is the non-intuitive interpretation of results wherein the absence of a band indicates the presence of a hemoglobin. There is concern that widespread use of these devices in the hands of users that are not properly trained may result in significant problems in test interpretation, possibly identifying many as having sickle cell disease or missing those that are truly HbSS. Further field testing will be important to determine the accuracy and reliability of the test results when performed and interpreted by untrained or minimally trained end-users.

Paper-based Rapid Diagnostic Test—The insolubility of deoxy-HbS in high concentrations of phosphate buffer is well described and is the physiological premise for the widely used sickle solubility test.[30, 31] In the presence of a highly concentrated phosphate buffer, deoxy-HbS polymerizes and precipitates, resulting in a clouded solution and visibly “positive” test which signifies the presence of HbS, but does not distinguish the carrier HbAS state from the homozygous HbSS state. Building upon this physiological principle, a paper-based and visually interpreted POC test (Halcyon Biomedical Inc., Friendswood, TX,

USA) has been developed. In this test, a small amount of whole blood (20 μ L) is mixed with a highly concentrated phosphate buffer containing hemolytic and reducing agents and added to a paper substrate. The polymerized HbS becomes entangled in the paper meshwork and remains in the center of the blood stain, while the soluble hemoglobins (HbA, HbF, HbC) wick laterally and create a wider blood stain on the paper. The result is interpreted based upon the differential intensity of polymerized HbS in the center region and soluble Hb in the peripheral region of the blood stain (Figure 3). Initial development of the POC test used automated measurement of color intensity using scanned images to correlate the blood stain pattern with the concentration of Hb S present, but the end product is designed to be interpreted visually.[32]

The test was validated in both a controlled laboratory setting on 55 samples including HbAA, HbAS, and HbSS and in the field in a sample of 226 pregnant women in Cabinda, Angola. The test demonstrated high sensitivity (93%) and specificity (94%) in the identification of HbSS samples (i.e. distinguish HbSS from HbAA and HbAS).[33] The test takes 30 minutes to complete and the estimated cost is \$0.07 for consumable reagents per test.

There are several limitations of the assay, including the inability to distinguish HbAS (trait) from HbSC (disease), as these conditions both have a similar percentage of HbS. The test discriminates the presence of HbS only and is not able to specifically identify the presence or absence of HbF, HbA, HbC, or other hemoglobin variants. The turnaround time for the current version of the test is reported to be 35 minutes, which is still improved from current assays but may be too long in certain busy clinical settings. The most important limitation of this test as it relates to its role in low resource settings is its lack of applicability for newborns and infants due to the predominance of fetal hemoglobin. The assay's limit of detection for Hb S is 20% and Hb S will not be detected in newborns who have a majority of Hb F. This limitation is important as early diagnosis is a critical first step in reducing morbidity and early mortality. The developers are working to develop a neonate-specific version of the test that is able to distinguish low percentages of HbS even in the presence of HbF. Secondly, the test is unable to distinguish the presence of HbC and thus cannot distinguish HbSC disease from the HbAS carrier state. As with all other POC tests, this assay requires further field evaluation to confirm that the visual interpretation of the blood stain is reliable and accurate.

HemeChip—Another potential POC device that holds promise for SCD diagnosis is the HemeChip, a portable microchip electrophoresis assay (Hemex Health Inc., Portland, OR) for detection and quantification of normal and variant hemoglobins. The assay is a miniaturized version of cellulose acetate electrophoresis, comprised of a micro-engineered plastic chip within a battery-powered electric field that separates various hemoglobin types based on charge.[34]

A small amount of blood from a finger stick (or heel stick in newborns), is mixed with deionized water. Then, a small amount ($< 1 \mu$ L) of the hemolysate is blotted onto the cellulose paper strip inside the chip and an electric field is applied across internal electrodes. Hemoglobins differentially migrate on the paper strip and separate based on charge to form

visibly distinct bands. In a laboratory pilot study, the HemeChip assay accurately and reliably identified Hb F, A, S, C/A2 and distinguished Hb phenotypes, including HbSS (HbS only), transfused HbSS (HbS and HbA), and Hb SC (HbS and HbC). The pilot study reported 89% sensitivity and 86% specificity for identification of Hb A, Hb S, Hb C/A2, and Hb F.[35] The test results were validated against standard electrophoresis and HPLC with excellent correlation for all Hb types tested. The LoD of this assay was assessed using adult blood samples and found to be 10% for HbS, HbF, and HbA, and 3% for HbC/A2.

The HemeChip device yields results in less than 10 minutes. Hemoglobin fractions displayed on the device can also be quantified using a smartphone image processing application. Including disposables and the reader, the cost per test is estimated to be \$2. As with standard cellulose acetate electrophoresis, the potential limitation of the assay is the inability to distinguish co-migrating hemoglobins, such as Hb C and A2. Although the device has not been tested in newborns, elevated levels of Hb F may mask the presence of very small amounts of other hemoglobins, such as Hb S and A. In addition, the HemeChip prototype currently uses a bench-top power supply with planned replacement by portable rechargeable batteries for POC applications. Field testing for clinical validation of the HemeChip POC device is planned this year.

SCD-AMPS-2 and SCD-AMPS-3—Sickled erythrocytes, which are formed in states of cellular dehydration, are denser than non-sickled cells. Two rapid tests have been developed by the same group (Daktari Diagnostics Inc., Cambridge, MA, USA) using detection methods relying upon the differences in density between sickled and non-sickled RBCs. These tests use aqueous multiphase systems (AMPs), which are mixtures of polymers in water that allow for separation of and discrimination between particles by density. The density of erythrocytes (comparable to the number of sickled cells) is greatest for homozygous HbSS or HbS β^0 -thalassemia with intermediate density in the compound heterozygous HbSC or HbS β^+ -thalassemia states, and the lowest for non-sickling conditions. Of note, there is variability in the number of dense cells for each genotype. The simpler version of the test, called SCD-AMPS-2, includes two fluid phases (one liquid-liquid and one liquid-container phase), while the more advanced SCD-AMPS-3 includes three phases (two liquid-liquid and one liquid-container) to separate and distinguish SCD and normal RBCs (Figure 4). The test requires the addition of ~5 μ L of whole blood to a preloaded plastic capillary with 14 μ L of reagents. This solution requires centrifugation (13,700 $\times$ g for 10 minutes) to adequately separate RBCs. To account for the lack of centrifuge equipment in limited resource settings, the authors used a microhematocrit centrifuge that can be powered by battery. After centrifugation, dense sickled cells were identified as those cells packed against the capillary at the bottom of the tube.

This test was evaluated using 59 samples with known genotype (26 HbAA, 7 HbAS, 20 HbSS, and 6 HbSC). The SCD-AMPS-2 test had a sensitivity of 90% and a specificity of 97% for the distinction of SCD samples (HbSS and HbSC) from non-SCD samples (HbAA and HbAS). SCD-AMPS-3 had similar sensitivity (91%) and specificity (88%), but was able to better distinguish HbSS from HbSC (sensitivity=67%, specificity=100%).[36] For both tests, samples from patients with sickle cell trait (HbAS) provide the same visual result as those from normal individuals, indicating that the test is able to diagnose SCD but cannot

distinguish between HbAA and HbAS. Additionally, the protective effect of HbF upon sickling reduces the number of dense cells, reducing the ability of the test to diagnose SCD (HbFS or HbFSC) in the newborn period. The SCD-AMPS-2 and SCD-AMPS-3 devices were evaluated in the field in a large (n=505) case-control study in Zambia. The sample size and distribution was designed to achieve approximately 50% SCD positive participants. The tests were shipped on ice and kept at 4°C until the day of testing. Compared to the testing performed in the laboratory, field testing demonstrated reduced performance with sensitivity of 87% and 75%, a specificity of 60% and 60%, and overall diagnostic accuracy of 77% and 69% for SCD-AMPS-2 and SCD-AMPS-3, respectively. Advantages of the test include rapid turnaround with results available within 12 minutes and low cost (estimated to be \$0.50 per test). The device appears relatively easy to use with a reported ease of use score of 3.4 on a scale from 1 (“very difficult to use”) to 5 (“very easy to use”). The authors suggest that the possible causes of reduced performance include bias and inexperience of the local Zambian readers, storage and shipping conditions, variability between batches of AMPS, and clotting of blood samples.[37] Potential sources of the high false positive rates observed, include clotting, variation between batches of SCD-AMPS, and shipping conditions.

Limitations of this test relate primarily to the dependence on red cell density and sickling to assign a test result. The test therefore has limited utility for the detection of SCD in newborns. Also, although the test detects the two common forms of sickle cell disease (HbSS and HbSC), it does not distinguish normal (Hb AA) from the carrier state (HbAS), which would limit its utility (to screen for reproductive risk) for premarital or community-wide screening programs. The field study conducted in Zambia highlights the importance of evaluating the end user experience with the devices. Variables potentially reducing the sensitivity and specificity of a rapid test may be related to the tests themselves (e.g., dehydration could affect the assay) or to differences in operators (e.g., less experienced readers may have a lower diagnostic accuracy).

Smartphone Based POC Test—Despite significant limitation in resources across the world, cell phones, even smartphones, remain nearly ubiquitous regardless of the presence or lack of resources. There is a surging interest in mobile health technologies in global health to reduce resource disparities and to optimize the provision of healthcare.[38–41] Given the decreased availability and high cost of laboratory and imaging diagnostics, there have been a number of mobile phone-based diagnostics developed over the past several years ranging from POC hemoglobin testing to POC ultrasound imaging. A recently described innovative technique uses a smartphone to distinguish the increased density of SS RBCs without the use of centrifugation or other advanced laboratory equipment. The test relies upon a smartphone attachment (named the Sickle Cell Tester) consisting of a 3D printed holder containing optical components, a battery to power an LED, and two permanent neodymium magnets containing a capillary for loading of the blood sample. The smartphone built-in camera captures the image of the microcapillary and the attachment magnifies and amplifies the image. In this assay, SS RBCs will demonstrate lower levitation heights when compared to control RBCs due to their increased density. A small quantity (<1 µL) of blood is mixed with sodium metabisulfite (to induce the deoxygenated form of hemoglobin) and gadolinium (to allow for magnetic levitation of RBCs) either by hand using a pipette or, as suggested by

the authors, simplified mixing using a finger-powered microfluidic mixing device. This sample is then loaded into a microcapillary and inserted into the SickLe Cell Tester and after a 10 minute period to allow for RBCs to magnetically equilibrate, the custom smartphone application rapidly analyzes (<1 second) the pixel intensity and location (Figure 5). The published report describing this novel technique analyzed 11 SS samples in comparison to 4 control samples (HbAA) and demonstrated clear and repeatable differences in the magnetic levitation properties of RBCs in persons with HbSS compared to HbAA samples.[42] This technique is promising, though the published data is preliminary and has not demonstrated the ability to differentiate between the homozygous HbSS and heterozygous HbAS state. In addition, the highly technical aspects of the smartphone attachment raises concern for its sustainability in the field, particularly related to hardware or software troubleshooting. The authors discuss the low cost of the reagents, but there is no discussion of the cost for the smartphone attachment and associated software.

CONCLUSIONS

With improvements in many other areas of global health, the recognition of SCD as an important global health problem is beginning to rise. Early identification of SCD, primarily through NBS programs, will significantly reduce the high early mortality of SCD within limited resource settings, but widespread early diagnosis is not practical with current diagnostic methods. The current standard laboratory diagnostic methods are expensive, require highly technical equipment and trained laboratory personnel and are thus not practical for widespread use in sub-Saharan Africa and other limited resource settings. Rapid, simple, and inexpensive tests able to provide an accurate diagnosis at the POC are desperately needed and could change the paradigm of SCD in limited resource settings where the burden of SCD is the greatest. As described throughout this manuscript, there are a number of promising POC assays in development for SCD. Table 2 summarizes the important features, benefits, limitations, and potential cost of the published POC devices for SCD. Each of the described tests has their own set of benefits and limitations, but all of them still require on the ground testing in the hands of the end user working in the field in high prevalence, limited resource settings. For a test to be practical in limited resource settings, the test should be able to be administered and reliably interpreted by untrained individuals with little or no training. The reagents and test devices must be robust and able to withstand harsh environments with high heat and humidity. Most of the tests described here have been validated in a very controlled US research laboratory but have yet to withstand the rigors of use in the field in sub-Saharan Africa. Before wide scale distribution and use, these field experiments must be performed with feedback from the end users in Africa to optimize the utility of the POC device in the settings where they will be used the most. In addition, the cost of these devices also needs to be optimized to be practical in settings where financial health resources are extremely limited. The developers of these POC assays are small businesses and are looking for at least a small profit margin. In order to facilitate widescale distribution, it may be necessary for larger philanthropic or global health organizations to subsidize or help to facilitate the distribution of these POC diagnostics in order to make them more widely available where they are needed the most.

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Abbreviations

AMPs	aqueous multiphase systems
CZE	capillary zone electrophoresis
IEF	isoelectric focusing
HPLC	high-performance liquid chromatography
NBS	newborn screening
POC	point-of-care
SCD	sickle cell disease
WHO	World Health Organization

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Figure 1. Sickie SCAN: A qualitative lateral flow assay

Steps of the Sickie SCAN point-of-care assay. The Sickie SCAN assay is a rapid and point-of-care device performed easily in several minutes. First, 5 μ L of blood (either from the finger as depicted in Panel A or pipetted from a previously collected sample) is added to 1.0 mL of prefilled buffer solution (Panels B and C). The tube containing the sample and buffer solution is mixed well by inverting three times (Panel D) and after discarding 3 drops, 5 drops of the lysate are added to the application pad on the Sickie SCAN cartridge (Panel E). The result is often visible within 1 min, but the final result is scored after 5 min with a darkened blue band indicating the presence of each hemoglobin. Panel F displays results for common hemoglobin patterns. Adapted from McGann PT et al, *Am J Hematol.* 2016 Feb 1;91(2):205–10.

Figure 2. HemoTypeSC™: A competitive lateral flow immunoassay

Panel A illustrates the design of the assay with HbS (●) and colloidal gold particles conjugated to HbA (■), HbS (●), BSA-C (▲), and an irrelevant mouse immunoglobulin (*). The test strip includes anti-HbS, anti-HbA, and anti-HbC Abs monoclonal antibodies and a goat anti-mouse control immunoglobulin. Panel B illustrates the flow of the sample through the test strip and Panel C shows the schematic of appearance of test results for each relevant phenotype. From Quinn CT et al. *Br J Haematol.* 2016.

Figure 3. Sickle Dx: A paper-based rapid test for SCD

The top panel is a schematic illustration of the basic principle behind the paper-based SCD assay. Schematic illustration of the basic principle behind the paper-based SCD assay. After mixing The bottom panel illustrates the expected results for common hemoglobin patterns. Note that samples containing polymerized Hb S become entangled in the center of the blood stain, resulting in a dark red spot, while soluble types of Hb (*e.g.* A, F, C) wick laterally through the paper substrate towards the periphery, coloring the paper faint red. From Yang X, et al. *Lab on a chip*. 2013;13(8):1464–7.

Figure 4. SCD-AMPS-2 and SCD-AMPS-3: Density-based SCD assays

Representative examples of positive and negative tests for the SCD-AMPS-2 (top) and SCD-AMPS-3 rapid tests demonstrate a clear distinction between subjects with (HbSC and HbSS) and without SCD (HbAA and HbAS). From Kumar AA et al. *PNAS* 2014;111:14864-14869.

Figure 5. Sickie Cell Tester: A smartphone based POC Device

Panel a and b are schematic diagrams of the lightweight smartphone attachment of the Sickie Cell Tester and included components and panels d through f are images of the actual platform. Panel c and g show sample captured images for this smart-phone based test. For comparison, panel h shows an example of a similar smartphone using a light microscope instead of the smaller smartphone platform. From Knowlton SM et al. *Sci Rep.* 2015;5:15022.

Table 1

	Equipment Cost (estimate)	Per test reagent cost (estimate)	Time
Citrate agar electrophoresis	~\$2,000	\$5	~1 hour of labor for one gel (14 samples) Labor: 2–3 hrs Dry gel overnight
IEF	~\$5,000	\$ 3.50	~8 hours of labor Labor: 2–3 hrs (incl reagent and gel prep, hemolysate prep) Gel has to dry overnight or oven required) for 44 samples
HPLC	~\$50–60,000	\$2–3	~8 hours of labor for 44 samples
CZE	~\$80,000	\$9/test	1 hour labor for each batch (20–30 samples) 3–4 hours (reagent preparation, need to run controls first, then check if okay, then run)

Table 2

Device Name	Method/Platform	Able to Distinguish				Able to Diagnose SCD in Newborns?
		SS from AS	SS from AA	SC from non-SC	AA from AS	
Sickle SCAN®	Lateral Flow	Yes	Yes	Yes	Yes	Yes
HemoTypeSC™	Competitive lateral flow	Yes	Yes	Yes	Yes	Yes
Sickle Dx	Paper-based	Yes	Yes	No	Yes	No
SCD-AMPS	Density-based	Yes	Yes	No	No	No
Sickle Cell Tester	Smart-phone based	Unknown	Yes	Unknown	Unknown	No