Density-based separation in multiphase systems provides a simple method to identify sickle cell disease

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Although effective low-cost interventions exist, child mortality attributable to sickle cell disease (SCD) remains high in low-resource areas due, in large part, to the lack of accessible diagnostic methods. The presence of dense ($\rho > 1.120$ g/cm^3) cells is characteristic of SCD. The fluid, self-assembling step-gradients in density created by aqueous multiphase systems (AMPSs) identifies SCD by detecting dense cells. AMPSs separate different forms of red blood cells by density in a microhematocrit centrifuge and provide a visual means to distinguish individuals with SCD from those with normal hemoglobin or with nondisease, sickle-cell trait in under 12 min. Visual evaluation of a simple two-phase system identified the two main subclasses of SCD [homozygous (Hb SS) and heterozygous (Hb SC)] with a sensitivity of 90% (73–98%) and a specificity of 97% (86–100%). A three-phase system identified these two types of SCD with a sensitivity of 91% (78–98%) and a specificity of 88% (74–98%). This system could also distinguish between Hb SS and Hb SC. To the authors' knowledge, this test demonstrates the first separation of cells by density with AMPSs, and the usefulness of AMPSs in point-of-care diagnostic hematology.

polymers | erythrocytes | sickle cell anemia | cell sorting | density gradient centrifugation

More than 300,000 children (~1% of births) are born with sickle cell disease (SCD) in Africa each year (1, 2). SCD is a genetic disorder caused by an array of genotypes [e.g., homozygous sickle cell disease (Hb SS) and hemoglobin SC disease (Hb SC)] that lead to the sickling of erythrocytes and associated pathologies (Table 1). Severity and specific symptoms vary between the subtypes of SCD; the most prevalent form (Hb SS, ~75%) is associated with the most severe effects (3).

Children with SCD suffer high mortality due to acute vaso-occlusive crises and increased risk of bacteremia (4, 5). Although inexpensive interventions exist to limit infection (e.g., penicillin prophylaxis, vaccinations) and reduce childhood mortality, more than 50% of children <5 y of age die in low-resource areas (4, 6, 7). Much of this mortality could be avoided, but to implement simple interventions, a diagnosis is necessary (8, 9). Standard diagnostic procedures, such as hemoglobin electrophoresis (HE) or HPLC, which are used in well-equipped facilities to detect SCD, are unfeasible in low-resource settings—where the disease is highly prevalent—and simpler methods, such as solubility tests, do not differentiate between SCD and the nondisease, sickle cell trait (Hb AS). This unmet need has motivated the recent development of creative diagnostic methods for SCD using blood stains on paper devices (10) and hemolysis in solutions of sucrose (11).

The distribution of density of red blood cells provides a biophysical indicator that is closely related to SCD pathophysiology (12, 13); the dehydration that leads to the formation of sickled cells increases the rate of the polymerization of hemoglobin S. Dehydration increases the density of the cell by reducing the volume and increasing the ratio of dense protein to less dense water in the cell. Although many of the cells with the highest density are also sickled, some may also have irregular shapes variously

described as a holly leaf or wreath shape (14–16). For simplicity, we refer to the entire class of high density cells characteristic of SCD as "dense, SCD cells."

This paper describes two rapid tests for the most prevalent forms of SCD (Hb SS and Hb SC) based on a sensitive but convenient measurement of the density (ρ, g/cm^3) of red blood cells using aqueous multiphase systems (AMPSs)—mixtures of polymers in water that form immiscible phases (previously referred to as MuPSs) (17). AMPSs provide a method of separating particles by density; the discrimination between particles of different density using an AMPS can be high ($\Delta\rho < 0.001$ g/cm^3) (17). Each phase of an AMPS is separated by an interface that defines a step in density. AMPSs are thermodynamically stable and reform if disturbed by stirring or shaking. AMPSs can be designed to be biocompatible, and have been used to separate mammalian cells by surface interactions (18, 19). To the authors' knowledge, this work presents the first use of AMPSs to separate cells based on their density.

We designed two AMPSs to separate erythrocytes into multiple bins of density; the presence or absence of erythrocytes in the bins distinguishes individuals with the most prevalent forms of SCD from individuals with either normal hemoglobin (Hb AA) or sickle-cell trait (Hb AS). The simpler test, SCD-AMPS-2, uses two phases; the higher-resolution test, SCD-AMPS-3, uses three phases. We evaluated our tests both visually and digitally in a population of 59 subjects [33 negative (Hb AA or Hb AS) and 26 positive (Hb SS or Hb SC)]. Both tests identified SCD positive samples with a sensitivity >90% and a specificity >88%. Details of the design and contents of both SCD-AMPS are provided in *Materials and Methods*.

Significance

Red blood cells with a high density ($\rho > 1.120$ g/cm^3) are characteristic of sickle cell disease. This paper demonstrates a density-based separation of red blood cells in a system of aqueous multiphase polymers that enables a visual test that identifies sickle cell disease, starting from samples of whole blood, in less than 12 min. This low-cost, simple test could provide a means to enable diagnosis of sickle cell disease in low-resource settings and enable life-saving interventions for children with the disease. The method itself provides a demonstration of the use of a biophysical indicator (here, density) rather than a biochemical marker (e.g., proteins separated by gel electrophoresis) as a means to do point-of-care hematology.

Table 1. Genetic disorders classified as SCD

Genotype	Description	Estimated annual births worldwide*
Hb SS	Homozygosity for Hb S	217,331
Hb SC	Heterozygosity for Hb S and Hb C	54,736
Hb S$\beta^{+/0}$	Heterozygosity for Hb S with concurrent beta thalassemia (0 or +)	11,074
Hb SE	Heterozygosity for Hb S and Hb E	N/A
Hb SD	Heterozygosity for Hb S and Hb D Punjab	N/A
Hb SO	Heterozygosity for Hb S and Hb O Arab	N/A

N/A, not applicable.
*Estimates based on literature (3, 20).

SCD Refers to a Large Number of Genetic Variations

Establishing whether the presence of dense cells at an interface of an AMPS is diagnostic of SCD requires testing many genetic variations of SCD as well as concurrent conditions that may affect the density of cells. *SI Appendix*, Table S1 lists several conditions that could affect the number of dense cells present in SCD. Homozygous sickle cell disease (Hb SS) and sickle cell with concurrent beta-0-thalassemia (Hb Sβ^+) are the most severe forms, whereas Hb SC and sickle cell with concurrent beta-+-thalassemia (Hb Sβ^+) are generally milder forms of the disease. In all cases, sickling of cells occurs, but the total amount of dense cells present may differ.

Hemoglobin C disease (Hb CC)—much more rare and geographically isolated than SCD (1, 20–22)—also increases the mass density of erythrocytes (23), and constitutes a potentially confounding interpretation. The distribution of densities of erythrocytes, however, may provide discrimination between SCD and Hb CC. In Hb CC, the entire distribution of densities of erythrocytes shifts to a slightly higher density; reticulocytes in Hb CC are denser than those in Hb AA, but the high-density erythrocytes in Hb CC may be less dense than the densest cells in Hb SS (12). In SCD, erythrocytes that are not sickled remain at normal densities, and the erythrocytes exist in two populations: a high-density, often sickled, population (~10% of cells) and a lower-density, predominantly normocytic, population, which also comprises reticulocytes and the youngest erythrocytes (24, 25).

For a proof of principle, we chose a population of subjects that represented the most prevalent forms of SCD (Hb SS and Hb SC) and those that represented typical negatives (Hb AA and Hb AS).

Dense Cells Are Characteristic of SCD

Dense cells present in SCD have a mass density (ρ ~1.12 g/cm^3) that is higher than the most dense erythrocytes in healthy individuals (ρ_{max} ~1.10 g/cm^3) (12, 24–26); the difference in density between these cells makes SCD a candidate for a density-based diagnostic test.*

Although the density distribution of erythrocytes in SCD has been studied extensively, commonly used methods of separating cells by density are not suitable for use in field settings (*SI Appendix*). Methods that separate multiple populations require tediously layered gradient systems that are destroyed by agitation or mixing, and simple systems can only separate a single population. Centrifugation through AMPSs allows the separation of multiple populations in a thermodynamically stable (and thus simple to use) system. AMPSs agitated by vigorous shaking readily reform quickly (minutes) under centrifugation, or more slowly (hours) under gravity (18).

The Interfaces in AMPSs Provide Bins of Density to Separate Cells

The number of subpopulations of erythrocytes of interest determines the number of phases that should be used (*SI Appendix*). An AMPS with three fluid phases provides enough interfaces—two liquid–

*Specific values of density vary in the literature and may be dependent on the media (e.g., Percoll, stractan, or phthalate esters). The values cited here were found to be consistent in AMPSs.

liquid interfaces and a liquid–container interface—to separate the three populations of erythrocytes required in a test for SCD that can distinguish the major subtypes (Hb SS and Hb SC) of the disease (Fig. 1). An AMPS with two phases provides two well-defined interfaces: one liquid–liquid interface between the phases, and one liquid–container interface. These interfaces are sufficient to separate dense, SCD cells from cells in the normal range of densities of erythrocytes, and to provide a test for SCD. Like the three-phase system depicted in Fig. 1, the presence of red cells at the bottom of the tube indicates a positive test for SCD. The simplicity of interpreting only two phases comes at the cost of the ability to distinguish subtypes of SCD.

A Point-of-Care Test Imposes Cost and Time Constraints on the Assay Design

To demonstrate the potential use of AMPSs in point-of-care (POC) settings, we designed tests to use ~5 μL of blood (a volume easily obtained from a finger stick). This blood was added to a plastic capillary that had been preloaded with 14 μL of the SCD-AMPS-3 or SCD-AMPS-2 (Fig. 2). We sealed the capillaries with either a white, vinyl-based sealant (Critoseal; Leica) for ease of visual detection or epoxy for clarity when imaging tubes by transmission mode in a scanner. Volumes were dictated by the capacity of the capillaries (*SI Appendix*). The total cost of reagents and materials per test is ~$0.20 at this scale; when fabrication costs and packaging materials are accounted for, the cost per test is ~$0.50 (*SI Appendix*, Table S2).

In rural settings, patients may travel for a day to seek medical care, and follow-up is challenging. Tests that can be coupled to actionable information and counseling must be rapid (ideally under 30 min). To meet this condition, we used a microhematocrit centrifuge (CritSpin; Iris Sample Processing) to centrifuge samples at $13,700 \times g$; this centrifuge can perform 12 tests at a time, and with a simple DC-to-DC converter can be powered by a car battery. We could distinguish blood of individuals with SCD from normal
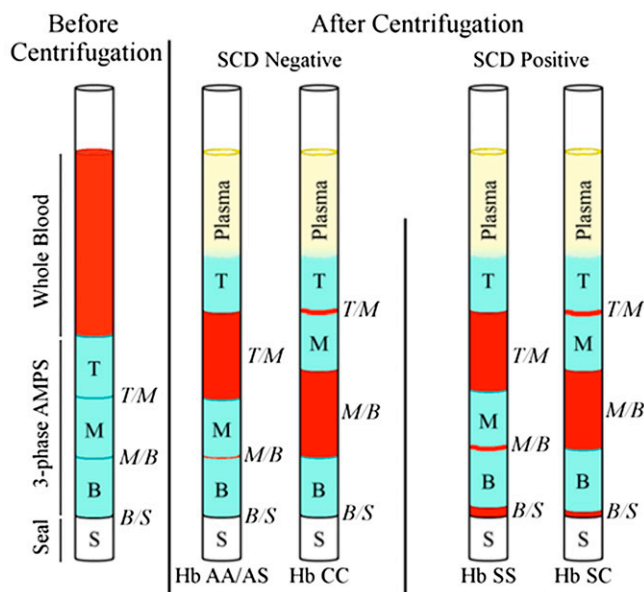

Fig. 1. Schematic representations for the four most important outcomes of a density-based rapid test for sickle-cell disease. Upon centrifugation, erythrocytes move through the top (T), middle (M), and bottom (B) phases of the AMPS and collect at some combination of the three lower interfaces (two liquid–liquid interfaces, *T/M* and *M/B*, and one liquid–container interface, *B/S*). The distribution of cells between these interfaces will depend on the genotype; nonsickle hemoglobins (Hb AA, Hb AS, and Hb CC) are distinct from sickle hemoglobins (Hb SS and Hb SC). In all cases, the presence of red at *B/S* indicates sickle cell disease. Three interfaces allow further discrimination; a majority of erythrocytes at *M/B* indicates Hb SC or Hb CC.

blood after 6 min; additional time in centrifugation enhanced the signal (*SI Appendix*, Fig. S1). We chose to use 10 min for our test to ensure a strong positive response, while keeping the test rapid. The rest of the procedure, including the finger-stick, sample loading, and test interpretation, took less than 2 min. By comparison, the gold standard for analysis of SCD, hemoglobin electrophoresis, requires more than 3 h to prepare samples, run electrophoresis, stain, and wash. Laboratories often batch samples to run on a single gel and report results several days later.

In addition, ease of use is a critical component of a POC test (27). Capillary action provides a simple mechanism to load blood. Designing a test to diagnose SCD from a finger-stick required a method to load blood into a capillary preloaded with SCD-AMPS and sealed on one end. Without modification, capillaries with closed ends will not wick additional fluid due to air trapped between the fluid and the sealed end. We used a "hole-in-tube" method to load blood into the capillary; puncturing a small hole in the side of the polycarbonate capillary at a specific distance

from one end allowed a standard volume of blood ($\sim$5 μL) to wick into the tube (*SI Appendix*, Fig. S2).

Results

Centrifugation of Blood Through the SCD-AMPS Provides a Visual Separation of Sickled Cells.
Over the course of 10 min of centrifugation at $13,700 \times g$, blood moved out of the loading zone and through the AMPS, and formed separated layers at the interfaces (Fig. 2). In most cases, the boundary between the top phase and the plasma was not distinguishable. The total packed volume between each interface provides an estimate of the hematocrit (*SI Appendix*, Fig. S3): this estimate also provides a simple, if crude, method to identify severe anemia concurrently (28). We could distinguish samples from individuals with SCD from normal samples with a diagnostic accuracy of over 90%; a layer of red cells sat below the bottom phase of both SCD-AMPSs and packed against the seal of the capillary (Fig. 3); this layer was dominated by sickled and dehydrated cells (*SI Appendix*, Fig. S6). By comparing the volume of the packed cells at the seal to the volume of cells at the other interfaces, we could estimate the percentage of dense cells (*SI Appendix*, Table S3).

In the SCD-AMPS-3, most erythrocytes within the normal density range sat at the upper liquid–liquid interface. The densest normal erythrocytes ($\sim$1–5% of erythrocytes) collected at the lower liquid–liquid interface. In both SCD-AMPS-2 and SCD-AMPS-3, blood from individuals with sickle-cell trait appeared the same as that from normal individuals; this method does not differentiate between Hb AS and Hb AA genotypes. Although a small amount of sickled cells may exist in blood from an individual with Hb AS, the amount is below our estimated limit of detection of 2.8% dense cells (*SI Appendix*, Fig. S7).

The Presence of Cells with a High Density Correlates with the Presence of SCD.
Samples that had a visible red band at the bottom of the AMPS correlated strongly with the presence of SCD in those with Hb SS or Hb SC. Conversely, samples negative for SCD rarely had red cells visibly present at the bottom of the AMPS (*SI Appendix*, Table S4). The SCD-AMPS-2 had a true positive rate (sensitivity) of 90% with a Jeffreys 95% confidence interval (CI) of 73–98% and a true negative rate (specificity) of 97% (CI = 86–100%). The SCD-AMPS-3 had a sensitivity of 91% (CI = 78–98%) and a specificity of 88% (CI = 74–98%). Admittedly, visual inspection allows room for bias in a diagnostic test. To reduce biased evaluation, each test was evaluated independently by at least two people (see *SI Appendix* for more details). Samples negative for SCD included both Hb AA (n = 26 for SCD-AMPS-3, n = 24 for SCD-AMPS-2) and Hb AS (n = 7). Samples positive for SCD included Hb SS (n = 20 for SCD-AMPS-3, n = 15 for SCD-AMPS-2) and Hb SC (n = 6). The formulation of SCD-AMPS-2 was finalized after testing had begun on SCD-AMPS-3, and thus the former system was tested on fewer samples.

In about half of all tests where a clear red band was not present at the bottom of the tube, visual inspection showed a thin layer of white, yellow, or pink material. Evaluation of these layers by microscopy revealed that platelets had clumped together to form large aggregates (*SI Appendix*, Fig. S5). In some cases, these aggregates appeared to have captured a small number of both red and white blood cells. In samples without SCD, we believe these aggregates may occasionally capture enough red blood cells to create a false positive.

We also evaluated scanned images of the capillaries digitally. Varying the thresholds for the intensity of the red color at the bottom of the tube produced a receiver operating characteristic (ROC) curve (Fig. 4 and *SI Appendix*). Our sensitivity and specificity from visual evaluation was near the ROC curve for both tests; this finding suggests two interrelated procedures: (*i*) with proper training, the visual reading of the tests by health workers could match (and be checked by) the performance of the digital analysis; or (*ii*) with higher-technology phones, digital analysis could replace training of readers. The fact that these two methods
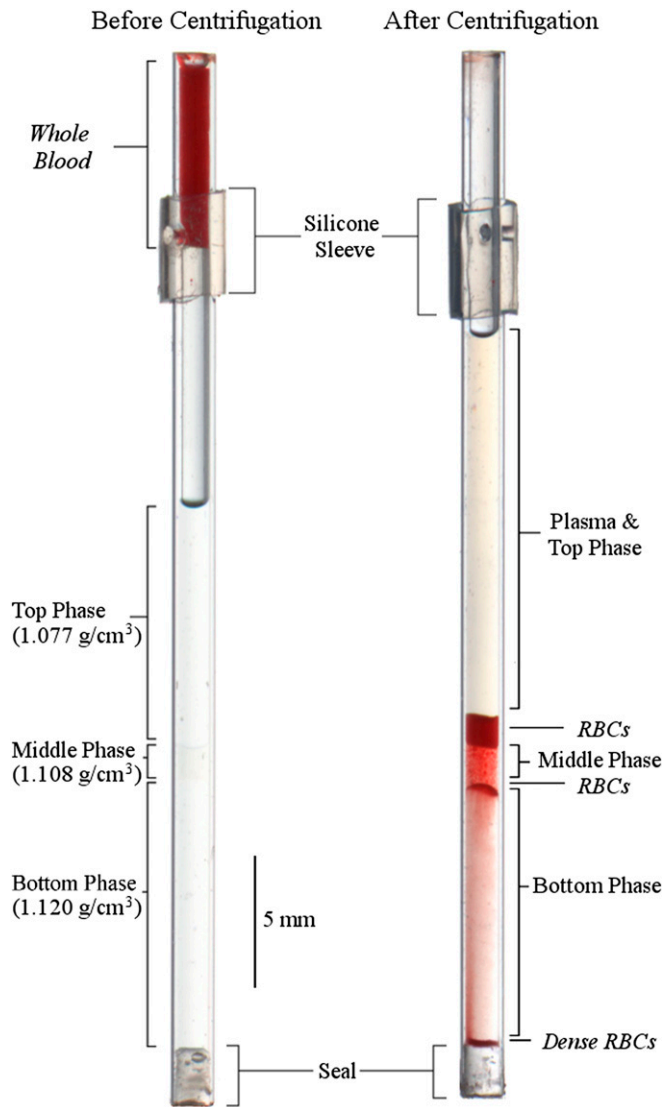

Fig. 2. Example of an SCD-AMPS-3 rapid test loaded before and after centrifugation of blood from a sample with sickle cell disease. Blood wicks just past the hole in the side of the tube that is then covered with the silicone sleeve. After centrifugation, the cells separate from the plasma and pack between the phases of the AMPS. The test shown would be classified SCD positive because there is red below the bottom phase and above the seal.
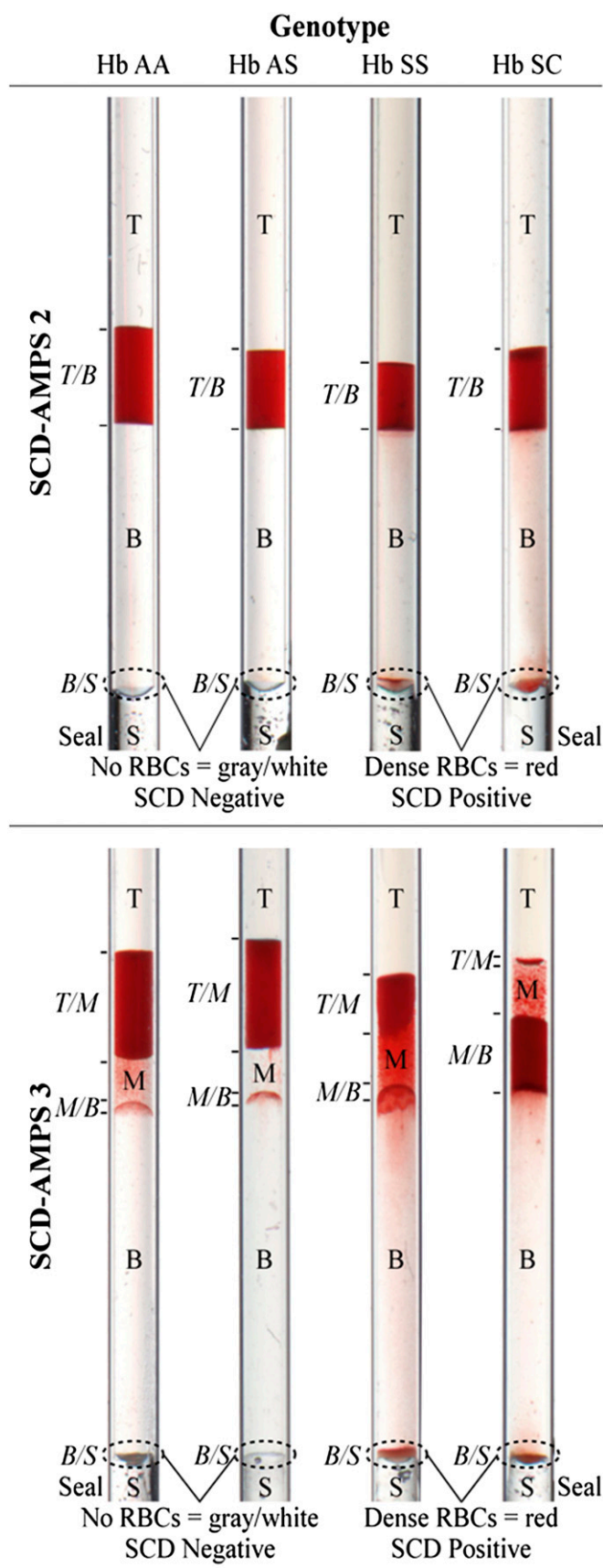
Fig. 3. Representative examples of positive and negative tests in the SCD-AMPS-3 (ρ_{top} = 1.077 g/cm^3, ρ_{mid} = 1.108 g/cm^3, ρ_{bot} = 1.120 g/cm^3) and the SCD-AMPS-2 (ρ_{top} = 1.078 g/cm^3, ρ_{bot} = 1.129 g/cm^3) show a clear distinction

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of reading give similar results suggest that reading the test will not be a major source of error.

The least-dense red blood cells in Hb SC have a slightly higher density than the least dense red blood cells in Hb SS (23). In SCD-AMPS-3, blood from most individuals with Hb SC had distributions of red cells distinct from those with Hb SS. In half the cases, a thick band of red cells formed at the lower liquid interface whose height was comparable to or greater than that of the band of red cells at the upper interface (Fig. 3). In some cases, an hourglass shape of red cells connected the packed cells at the two interfaces (*SI Appendix*, Fig. S8). By contrast, blood from samples with Hb SS had two distinct bands of red with a clear majority of red cells at the upper liquid interface. These differences in the distribution of cells allowed us to distinguish visually between Hb SC ($n = 6$) and Hb SS ($n = 20$), the two most prevalent forms of SCD with a sensitivity of 67% (CI = 29–92%) and a specificity of 100% (CI = 88–100%). Centrifugation for an additional 10 min increased the sensitivity to 83% (CI = 44–98%). In Fig. 3, the red and pink in the lower phases indicated either that the dense cells had not all reached their equilibrium position or that some cells had the same density as one of these phases. Additional centrifugation time (a total of 30 min) allowed these cells to sediment further and increased the thickness of the layer of red at the bottom of the tube (*SI Appendix*, Figs. S1 and S4), but required more time.

Discussion

An AMPS-Based Test for SCD Is Appropriate for Use at the POC. The World Health Organization (WHO) recommends that POC devices be affordable, sensitive, specific, user-friendly, reliable, equipment-free, and deliverable to those in need (27). AMPS-based tests for SCD have the characteristics of sensitivity, affordability, and ease of use required to have an impact at the POC.

The hole-in-tube method allows blood to fill the tube automatically. Unlike tests that require lysing and incubating blood in a solution (e.g., Sickledex), whole blood is tested directly. Minimal handling of the sample reduces errors and risks to health workers performing the test. We estimate the cost of materials and manufacturing per test to be $0.50 (*SI Appendix*). The centrifuge (CritSpin; Iris Sample Processing) we use in this study costs ~$1,600, but we have verified that our system performs similarly on a basic centrifuge (SpinCrit; www.spincritcentrifuge.com) that costs $150, is portable, and runs on four AA batteries; this centrifuge can perform six tests at a time, and standard AA batteries allow three separate 10-min spins per charge.

Although SCD-AMPS do not distinguish between Hb AS and Hb AA, they can distinguish between Hb AS and both Hb SS and Hb SC. SCD-AMPS-3 has the added ability to distinguish Hb SC from Hb SS, albeit with a lower sensitivity than that with which the test identifies SCD—the sensitivity to identify Hb SC may be improved with a slightly different density of the middle phase. In addition to providing diagnostic information, these tests measure a biophysical indicator that may help identify patients more likely to experience certain complication of the disease; the fraction of erythrocytes that have a high density correlates with certain clinical manifestations of SCD (e.g., skin ulcers, priapism, and

between subjects with SCD (Hb SS and Hb SC) and those without SCD (Hb AA and Hb AS). In non-SCD blood in the SCD-AMPS-2 system, all cells pack at the liquid interface (*T/B*). In the SCD-AMPS-3 system, most cells have normal morphologies and densities (normocytes) and pack at the upper liquid interface (*T/M*), the densest normal-shaped cells (dense normocytes) collect at the lower liquid interface (*M/B*), and some aggregated platelets are present at the bottom of the tube (gray; *B/S*). Erythrocytes from a subject with SCD display greater heterogeneity at high densities. Dense, SCD cells form a layer below the bottom phase of both SCD-AMPS on top of the sealant (*B/S*). In the SCD-AMPS-3 test, the distribution of cells between the liquid interfaces (*T/M* and *M/B*) differentiates Hb SS from Hb SC. The difference in the total packed volume of the cells demonstrates the difference in the hematocrit between the normal subject and the anemic subject with SCD.
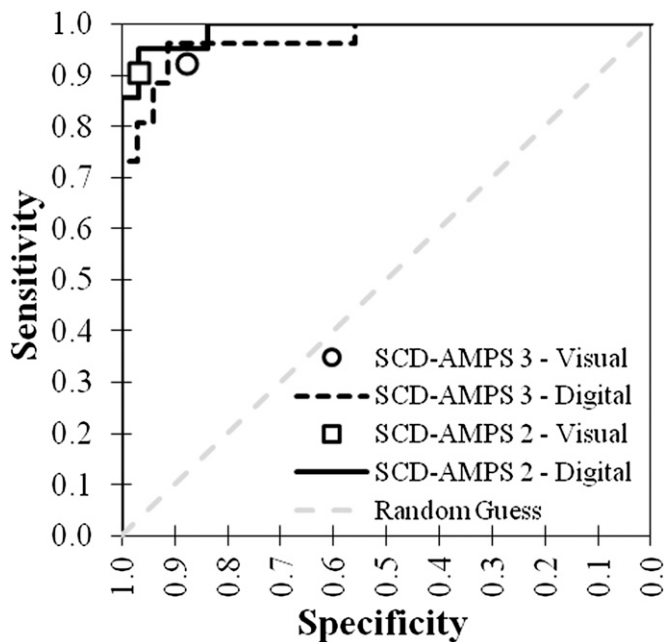
Fig. 4. The ROC curves (solid and dashed black lines) for the digital evaluation of the presence of a red layer at the bottom of the SCD-AMPS demonstrates good diagnostic performance. Both curves are far from the gray line that indicates no ability to detect an event. Visual evaluation of the rapid tests was near the sensitivity and specificity of the digital analysis.

renal dysfunction) (29). Monitoring the distribution of the density of cells could also provide a way to assess sickle crises (30).

The Density of Cells Can Be Used as a Diagnostic Marker. The use of density as a tool for POC diagnostics began with the spun hematocrit, in which the difference in density between cells and plasma enables the measurement of the volume ratio of packed cells in blood. This measure has been helpful in the diagnosis of anemia (28). Modifications on the spun hematocrit concept have led to systems for measuring white blood cell counts and other parameters (31). The company Zyomyx has developed a technique to tag CD4+ cells with proprietary high-density beads so that these cells can be separated from whole blood and quantified (32).

We have demonstrated, to our knowledge for the first time, that the self-assembling step-gradients in density formed by AMPSs can separate cells by density, and provide a new method to identify SCD. The presence of dense cells correlates with the presence of SCD (12); AMPSs may provide a simple tool to identify dense cells in low-resource settings. Further testing will be required to verify the performance of the SCD-AMPS on larger populations with more genetic variation and concurrent conditions, but this work demonstrates that density has the potential to be a sensitive and specific biophysical marker for diagnosing SCD.

One limitation of density-based methods is that density is a colligative property; density depends on the sum of the elements (solutes, proteins) contained in a cell rather than on a specific biochemical marker. As discussed earlier, factors that can affect the density of red blood cells, such as genetic variations and iron deficiency anemia (*SI Appendix*, Table S1), must be carefully assessed to develop a POC diagnostic based on density. Testing on a larger population that might include patients with these and other concomitant conditions would determine the generality of density as a diagnostic for SCD.

The densities of SCD-AMPS described here should discriminate between Hb CC and SCD; if, however, dense cells in Hb CC do provide false positives, an additional low-density band to isolate reticulocytes could discriminate Hb CC from Hb SC and Hb SS (23).

Density-based tests, also, will not be sensitive when dense cells are not present. At birth, children with SCD have predominantly Hb F, and generally begin to express appreciable amounts of Hb S between 6 mo and 1 y of age. In some cases, high levels of Hb F may persist in children until they are 2 y old. The presence of dense, SCD cells relies on some amount of dehydration or sickling of cells and, hence, on the presence of large amounts of Hb S. Screening young children with a high proportion under 2 y old might have lower sensitivity than the performance described in this study. Only one of our Hb SS samples came from a child under 1 y old, and it appeared negative on both digital and visual inspection. Developing an accessible and affordable screening test for newborns remains a critical, unmet challenge. Rapid diagnostics that rely on the presence of sickled cells, such as ours, might play an important role in reducing child mortality if screenings are done near a child's first birthday; such diagnostic efforts could be carried out simultaneously with vaccination campaigns (e.g., measles) that target children between 9 mo and 1 y old.

The percentage of dense, SCD cells in the blood varies among individuals. Factors that can affect the amount of dense cells present in the blood include hydration and the occurrence of a recent vaso-occlusive crisis (30). Under most conditions, however, the blood of individuals with SCD has 13% (SD 8%) dense cells (29). This estimate of the amount of dense cells present includes a large population and modulators that can reduce the amount of dense cells present, such as high levels of Hb F and concurrent α-thalassemia. In these individuals, sickled cells comprise a smaller proportion of erythrocytes, and clinical symptoms are normally milder than others with SCD (33, 34).

An AMPS-Based Test for SCD Can Fill a Gap in Global Health. With progress against infectious diseases around the world, the burden of morbidity and mortality due to hemoglobinopathies, such as SCD, will rise (3). Standard diagnostic techniques in well-equipped laboratories, such as HE and HPLC, are too expensive and require more infrastructure than is available in many countries with high burdens of SCD, especially in rural areas (35).

Sickling tests using a deoxygenating agent and microscopy are subject to error from preparation and interpretation (36). Solubility tests are among the most used, microscope-free methods for screening for SCD in low-resource settings (37). In these tests, a solution lyses and deoxygenates the blood; the polymerization of deoxyhemoglobin S causes the hemoglobin to form a nematic liquid crystal and makes the solution turbid (38, 39). Both sickling and solubility tests can detect the presence of Hb S but cannot distinguish between Hb SS and Hb AS without the use of additional, expensive equipment (e.g., a turbidimeter); this distinction is nontrivial, because the former is a life-threatening condition and the latter is largely benign.

In response to the outstanding need for rapid tests for SCD appropriate for the POC, a request for applications by the National Institutes of Health in 2013 solicited the creation of tests for SCD with a sensitivity of at least 60% (RFA-HL-14-010). The AMPS-based tests described here have a sensitivity of over 87% in the patient samples used for this initial validation study.

Compared with currently available techniques, the density-based tests using AMPS combine four desirable properties: (*i*) fieldability—they are amenable to use at the POC, (*ii*) performance—they can distinguish Hb SC and Hb SS from Hb AA or Hb AS, (*iii*) biophysical information—by quantifying the percentage of dense, SCD cells, and (*iv*) low cost. The AMPS-based method also compares favorably in ease of use to other technologies that have been developed recently to diagnose SCD (*SI Appendix*, Table S4).

The high sensitivity and specificity of SCD-AMPS in identifying SCD in tests in a laboratory described here demonstrates that a density-based approach may provide a valuable screening tool. Further testing on different genotypes and concurrent conditions will determine whether such a test is appropriate as a POC diagnostic for SCD. Even if the performance is reduced, the densities could be adjusted to provide a higher-sensitivity test

(with lower specificity) to be used as a screening method; such a test would allow interventions where the cost to treat is low.

By combining simplicity and rapidity to measure a biophysical parameter (i.e., density), the density-based test using AMPSs could play an important role in diagnosing SCD at the point of care. Measuring the fraction of dense cells at the bottom of an SCD-AMPS could also have use beyond the diagnosis of SCD, and possibly aid in the management of the disease, but such uses will require clinical validation. More generally, density-based diagnostics illustrate how biophysical markers, such as density, and simple separation methods, such as centrifugation through AMPSs, can combine to provide low-cost health solutions; AMPS-based separations of blood should enable hematology at the point of care.

Materials and Methods

Designing SCD-AMPS. When designing an AMPS, we first seek systems whose phases are separated by the same differences in density as our target subpopulations of erythrocytes. We then use other additives to tune the overall density to the necessary levels.

An AMPS formed by mixing 7.0% (wt/vol) PEG with a molecular weight (MW) of ~20 kDa and 10.3% (wt/vol) Ficoll with a MW of ~400 kDa provided phases with densities separated by the values required for a two-phase system useful in the diagnosis of SCD. An AMPS comprising 3% (wt/vol) PEG with a MW of ~20 kDa, 10% (wt/vol) dextran with a MW of ~500 kDa and 5% (wt/vol) polymer of partially hydrolyzed poly(vinyl acetate) (containing 75% −OH and 25% −OCOCH₃ groups) with a MW of ~3 kDa, provided phases separated by differences in density of the values required for our three-phase assay.

We added NaCl to make the system isotonic (as measured by vapor pressure osmometry) with blood. The pH of each system was adjusted to 7.40 ± 0.02 by the addition of concentrated (1–10 M) NaOH or HCl. We used a low-osmolality, high-density additive (Nycodenz; Accurate Chemical) to increase the density of each system to the proper range, and measured the density of each phase using an oscillatory U-tube densitometer. SCD-AMPS-2 contained 9.1% (wt/vol) Nycodenz and had phases with densities $\rho_{top} = 1.078$ g/cm³ and $\rho_{bot} = 1.129$ g/cm³. SCD-AMPS-3 contained 8.7% (wt/vol) Nycodenz and had phases with densities $\rho_{top} = 1.077$ g/cm³, $\rho_{mid} = 1.108$ g/cm³, and $\rho_{bot} = 1.120$ g/cm³.

SI Appendix. The *SI Appendix* includes detailed descriptions of all chemicals and materials used, methods for obtaining blood samples, the preparation and characterization of the AMPS, estimations of the cost per rapid test (*SI Appendix*, Table S2), analysis of test performance with different centrifugation times (*SI Appendix*, Fig. S1 and S4), experimental and rapid test design (*SI Appendix*, Fig. S2), estimation of hematocrit and fraction of dense cells (*SI Appendix*, Fig. S3 and Table S3), analysis of separated fractions of cells (*SI Appendix*, Figs. S5 and S6), comparison of methods to identify SCD (*SI Appendix*, Table S4), estimation of the limit of detection using cells treated with nystatin as a model for dense, sickled cells (*SI Appendix*, Fig. S7), and detailed results on clinical samples (*SI Appendix*, Fig. S8 and Table S4).

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