

# Lab on a Chip

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Chromatography Paper as a Low-Cost Medium for Accurate Spectrophotometric Assessment of  
Blood Hemoglobin Concentration



We investigate using chromatography paper as a low-cost medium for spectrophotometric assessment of blood hemoglobin concentration and present a prototype low-cost reader for field evaluation.

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Chromatography Paper as a Low-Cost Medium for Accurate Spectrophotometric Assessment of  
Blood Hemoglobin Concentration

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## ABSTRACT

Anemia affects a quarter of the world's population, and a lack of appropriate diagnostic tools often prevents treatment in low-resource settings. Though the HemoCue 201+ is an appropriate device for diagnosing anemia in low-resource settings, the high cost of disposables (\$0.99/test in Malawi) limits its availability. We investigated using spectrophotometric measurement of blood spotted on chromatography paper as a low-cost (<\$0.01/test) alternative to HemoCue cuvettes. For this evaluation, donor blood was diluted with plasma to simulate anemia, a micropipette spotted blood on paper, and a bench-top spectrophotometer validated the approach before the development of a low-cost reader. We optimized impregnating paper with chemicals to lyse red blood cells, paper type, drying time, wavelengths measured, and sensitivity to variations in volume of blood, and we validated our approach using patient samples. Lysing the blood cells with sodium deoxycholate dried in Whatman Chr4 chromatography paper gave repeatable results, and the absorbance difference between 528 nm and 656 nm was stable over time in measurements taken up to 10 min. after sample preparation. The method was insensitive to the amount of blood spotted on the paper over the range of 5  $\mu$ L to 25  $\mu$ L. We created a low-cost, handheld reader to measure the transmission of paper cuvettes at these optimal wavelengths. Training and validating our method with patient samples on both the spectrometer and the handheld reader showed that both devices are accurate to within 2 g/dL

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of the HemoCue device for 98% and 95% of samples, respectively.

## INTRODUCTION

Anemia affects a quarter of the world's population (1.62 billion people) and can be caused by iron deficiency, malnutrition, blood loss, and infectious diseases (e.g. malaria, hookworm, tuberculosis, HIV), among others<sup>1</sup>. Anemia can cause delayed mental and physical development, fatigue, decreased work productivity, and increased risk of mortality, especially during childbirth<sup>1</sup>. The condition is diagnosed by measuring the concentration of hemoglobin in the blood. The threshold hemoglobin concentration below which a patient is considered anemic varies by lab and patient condition, but is approximately 11 g/dL for young children and pregnant women, 12 g/dL for non-pregnant women, and 13 g/dL for men (WHO hemoglobin thresholds for people living at sea level)<sup>1</sup>. Once the condition is diagnosed, the underlying cause can be determined and treated with, for example, iron supplements, anti-malarial drugs, or blood transfusions.

Hemoglobin concentration assessment is the most commonly performed laboratory test worldwide and is necessary for all healthcare systems. Assessment at the point-of-care enables clinicians to make rapid decisions about treatment. The gold standard for measuring hemoglobin concentration converts hemoglobin to cyanmethemoglobin, a stable variant, and uses a spectrometer to measure its absorbance at 540 nm. This method is not appropriate for use at the point-of-care.

The HemoCue method draws blood into a plastic cuvette and makes spectroscopic measurements to return a hemoglobin concentration. A comprehensive assessment of six hemoglobin concentration tests in Malawi, taking into account accuracy and clinical usefulness, user friendliness, learning and usage time, and economic costs, revealed that HemoCue was the most appropriate method and the recommended standard-of-care in all Malawian district hospitals and urban health centers<sup>2</sup>. The HemoCue method has two associated costs: a fixed cost for the device (\$470 from MM African Technologies Ltd in Malawi or \$800 from HemoCue, Inc. in the U.S.) and a

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recurring cost for each cuvette (\$0.99 from MM African Technologies Ltd. in Malawi or \$1.43 from HemoCue, Inc. in the U.S.). Though the fixed cost is significant, it is primarily the high recurring cost of consumables that prevents widespread utilization of this accurate and easy-to-use hemoglobin concentration test in low-resource areas where anemia prevalence is highest<sup>2</sup>. The cost of just one cuvette (excluding treatment for anemia, if diagnosed) represents 3% of Malawi's per capita total expenditure on health per year (\$26 in 2010 at an average exchange rate, WHO Global Health Observatory Data Repository). Thus, there is a need for a low-cost alternative that can reduce the consumable component of the per-test cost of assessing hemoglobin concentration at the point-of-care.

### Chromatography Paper Alternative

Recently, filter or chromatography paper has been proposed as a low-cost, rugged, and self-contained medium for microfluidic assays in point-of-care systems suitable for developing countries<sup>3</sup>. The ubiquitous and low-cost nature of chromatography paper represents an attractive alternative to expensive plastic cuvettes used in systems such as HemoCue. The goal of this study is to investigate if chromatography paper can serve as a low-cost medium for accurate spectrophotometric detection of blood hemoglobin concentration. We further demonstrate a prototype low-cost device to measure transmission through this chromatography paper "cuvette" and display the hemoglobin concentration at the point-of-care.

### MATERIALS AND METHODS

In order to validate a paper-based spectrophotometric hemoglobin assessment, we performed experiments to optimize or evaluate sensitivity to five parameters: impregnating paper with

chemicals to lyse red blood cells, paper type, drying time, wavelengths measured, and volume of blood. Blood and plasma samples were obtained via venous draw from healthy donors who gave informed consent; plasma was also purchased from the Gulf Coast Regional Blood Bank (Houston, TX). Protocols were reviewed and approved by the Institutional Review Board at Rice University. For these experiments, whole blood was diluted with plasma to simulate anemia. Micropipettes were used to spot blood on paper to simulate touching a patient's fingertip to the paper after a fingerprick. A benchtop spectrophotometer (Cary 5000 UV-VIS) recorded the spectra of the samples via an on-axis collimated transmission measurement. The samples were masked with a 3 mm x 3 mm aperture. We used the HemoCue 201+ as a reference standard to develop and evaluate the paper-based approach. The HemoCue 201+ is CLIA (Clinical Laboratory Improvement Amendments) waived and approved for use in the United States by the FDA. HemoCue, Inc. reports an accuracy of  $\pm 1.5\%$  for the HemoCue 201+ when compared to the International Council for Hematology (ICSH) method. Gehring et al. have reported the HemoCue 201+ to be accurate to within approximately 0.5 g/dL of the reference method in a laboratory setting<sup>4</sup>. Paddle et al. have reported 95% limits of agreement of  $-1.16$  g/dL to  $0.16$  g/dL in a laboratory setting, and Patel et al. have reported a mean difference of  $0.110 \pm 0.524$  g/dL from the reference standard in a hospital setting<sup>6</sup>. Our own measurements provide evidence of the precision of the HemoCue. Values from the spectrum of blood spotted on paper were correlated with the hemoglobin concentration of the sample obtained with a HemoCue. Finally, using conclusions from experiments performed on the spectrometer, we developed a portable, low-cost reader to replace the spectrometer in the field.

### Red Blood Cell Lysis

We examined unlysed blood samples as well as samples where red blood cells were mechanically lysed or chemically lysed with sodium deoxycholate. To achieve mechanical lysis, unlysed blood was taken through multiple ( $\geq 3$ ) freeze-thaw cycles ( $-20^{\circ}\text{C}$  to  $20^{\circ}\text{C}$ ). The blood was considered lysed if high-speed centrifugation did not separate the blood into plasma and red blood cell layers and microscopy showed no intact cells.

To achieve chemical lysis of blood spotted on Whatman Chr4 paper, we used the detergent sodium deoxycholate. To evaluate the amount of sodium deoxycholate needed to achieve lysis on paper, paper strips were treated with various volumes (10, 20, 30, 40, and 50  $\mu\text{L}$ ) of 2% or 4% (w/v) sodium deoxycholate in PBS. After the sodium deoxycholate dried, 10  $\mu\text{L}$  of unlysed blood was applied to the treated paper. Unlysed blood and mechanically lysed blood applied to untreated paper served as controls. The blood dried for 2 min and was then eluted from the paper in 1 mL PBS for 10 min. The paper was removed and the spectrum of the remaining solution was measured on a Cary 5000 UV/VIS spectrophotometer from 450 nm to 800 nm. Blood was considered lysed if its transmission spectra did not show evidence of the turbidity associated with intact red blood cells. 10  $\mu\text{L}$  of 4% (w/v) sodium deoxycholate was chosen for all further experiments because (1) it resulted in effective lysis and (2) the blood spread quickly and evenly on the paper without pooling.

To assess the effect of red blood cell lysis on the accuracy of hemoglobin concentration derived from spectral measurements, we measured transmission spectra of samples with varying concentrations of hemoglobin for three conditions: unlysed blood applied to untreated Chr4 paper, mechanically lysed blood applied to untreated Chr4 paper, and unlysed blood applied to sodium deoxycholate-treated Chr4 paper. Blood from 4 donors was diluted with plasma to obtain a range of hemoglobin concentrations spanning the physiologic range. The experiment was repeated on 2 days for each condition; half of the data were used as a training set, and the remaining data were used as a



validation set. The training set for each condition was used to develop an algorithm relating hemoglobin concentration as measured by HemoCue to the absorbance difference at two wavelengths as described in detail in the section “Training and Validation with Patient Samples.” This relationship was used to calculate the hemoglobin concentration of samples in each validation set, and the calculated hemoglobin concentrations were compared to the concentrations obtained from HemoCue. The deviations from HemoCue were binned in 0.1 g/dL increments, and the cumulative percentage of samples with a given deviation was plotted for the three conditions.

### Choice of Paper

Seven filter and chromatography papers from Whatman (chromatography papers Chr1, Chr3MM, Chr4, and grades 2, 4, 5, and 6 filter paper) were evaluated for (1) the qualitative appearance of uniform spreading of unlysed blood spotted on sodium deoxycholate-treated paper and (2) the repeatability of spectrophotometric measurements of these blood samples with high and low hemoglobin concentrations. Paper strips were treated with 10  $\mu$ L 4% (w/v) sodium deoxycholate in PBS to achieve lysis and allowed to dry. 10  $\mu$ L of unlysed blood was applied and spectra were taken 2 min after spotting. For each type of paper, three measurements were made of blood with a low hemoglobin concentration (7-8 g/dL) and three of blood with a high hemoglobin concentration (15-16 g/dL). Among the papers where blood spread quickly and with little pooling, the measurements of Chr4 had the most repeatable measurements (coefficient of variation of 0.04 for low hemoglobin concentrations and 0.06 for high hemoglobin concentrations) (data not shown). Chr4 was used for all other experiments.

### Choice of Wavelength and Time

We determined which spectral measurements could be used to calculate consistent hemoglobin concentrations even if the drying time of the spot varied, an important factor for point-of-care use. To determine the effects of drying time and to select optimal wavelengths for determining hemoglobin concentration, absorption spectra of unlysed blood spotted on sodium deoxycholate-treated Chr4 paper were collected from 450 nm to 800 nm over the course of 30 min.: the time during which a hemoglobin concentration assessment could reasonably be performed in the field. Blood from one donor was diluted with plasma to obtain four hemoglobin concentrations approximating the physiological range. Each concentration was measured three times, and spectra were taken every 2 min.

### **Effects of Blood Volume**

Absorption spectra of various volumes (5, 10, 15, 20, 25  $\mu\text{L}$ ) of unlysed blood on sodium deoxycholate-treated Chr4 paper were obtained to determine the effects of blood volume on the test. Three hemoglobin concentrations were examined, and three measurements were made of each concentration at each volume. These transmission spectra were used to calculate hemoglobin concentrations with an algorithm developed using data from a training set of samples described in the section “Training and Validation with Patient Samples;” we examined whether the calculated concentration of hemoglobin depended on sample volume.

### **Training and Validation with Patient Samples**

An algorithm was developed to calculate hemoglobin concentration from on-axis transmission measurement of blood spotted on paper. Patient blood samples were used to train and validate this algorithm on the laboratory spectrometer. Whole blood samples were obtained from 48

hospitalized patients. Venous blood was collected in heparinized tubes; anonymous specimens were obtained one week after collection. The protocol was reviewed and approved by the Rice University IRB and was found to be exempt from IRB review. 5 patient samples were discarded because the blood showed significant clotting. 2 measurements were made from each patient sample. Data from 21 patients (42 measurements) were used to develop an algorithm relating the hemoglobin concentration as measured by HemoCue to the absorbance difference between two wavelengths using a best-fit power curve:

$$[\text{Hb}] \text{ in g/dL} = A * [\text{Extinction coefficient}(\lambda_1) - \text{extinction coefficient}(\lambda_2)]^n$$

where  $A$ ,  $\lambda_1$ ,  $\lambda_2$ , and  $n$  were varied.

These wavelengths include one wavelength from 450 nm to 600 nm where hemoglobin absorbs and one wavelength from 600 nm to 800 nm where it does not absorb. As described in the results section, the pair of wavelengths that gave the best results and was least sensitive to variations in time between sample preparation and sample measurement was 528 nm and 656 nm. Data from the remaining 22 patients (44 measurements) were used to validate the performance of this algorithm. The hemoglobin concentration for these samples was calculated from the transmission data using the relationship from the training set; results were compared to those measured by the HemoCue. For both training and validation sets, a reading was taken on the spectrometer at 2 min. after spotting.

### Development of a Low-Cost Reader

Following validation of the algorithm to calculate hemoglobin concentration on a spectrometer, we developed a prototype low-cost reader to replace the spectrometer (Figure 1). A diagram of the optical components of the low-cost reader is presented in Figure 1b. Light emitted from two LED diodes (HyperRed,  $\lambda = 656 \pm 25$  nm, 720-LHW5AM1T3T1LZ from Osram via

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Mouser Electronics and TrueGreen,  $\lambda = 528 \pm 33$  nm, 720-LTW5SMJXKX36Z from Osram via Mouser Electronics) is collimated by plastic aspheric lenses (EFL=3.3mm, NA=0.4, CAY033 from ThorLabs) and, after passing through 50/50 beamsplitter (BS007 from ThorLabs), is directed onto the sample. Light transmitted through the sample is detected by a broad-band photodiode (wavelength range: 350-1100 nm, FDS100 from ThorLabs) placed behind the sample chamber. All electronic components of the system are connected to an Arduino Nano 3.0 microcontroller. A custom-written Arduino program was used to acquire and analyze measurement data. Measurements consist of a 2 sec. baseline reading with both LEDs off to enable subtraction of ambient light, a 2 sec. reading with the 528 nm LED on, and a 2 sec. reading with the 656 nm LED on. Results are displayed on a three digit numeric display (Kingbright BC56-12SRWA), and the prototype is powered by a rechargeable 9V battery. The device measures 6.4 cm x 6.6 cm x 7.8 cm and weighs 281 g.

Samples are inserted between two custom 3D-printed plastic pieces to isolate the reader from biohazards. These pieces may be reused for many samples and are sized such that they may be replaced by glass slides. The chromatography paper is cut such that aligning it with the depression in the plastic holder ensures proper alignment of the blood sample with the optics of the low-cost reader.

Data were collected from the same samples used to train and validate the spectrometer at 90 sec. after spotting. Data from the training set were used to develop an algorithm relating the difference in transmission at 528 nm and 656 nm to the hemoglobin concentration as determined by the HemoCue:

$$[\text{Hb}] \text{ in g/dL} = A * [\text{Extinction coefficient}(\lambda_1) - \text{extinction coefficient}(\lambda_2)]^n$$

where A,  $\lambda_1$ ,  $\lambda_2$ , and n were varied.

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The algorithm was then used to predict the hemoglobin concentration for the samples in the validation set using the concentration determined with the HemoCue as the gold standard. The Arduino program was developed to calculate and display the hemoglobin concentration according to this algorithm.

## RESULTS AND DISCUSSION

**Absorbance Spectrum of Blood on Paper**

From 450 nm to 600 nm, spectra of blood on paper show characteristic absorbance peaks due to hemoglobin; above 600 nm, spectra show a nearly flat baseline region (Figure 2). These spectra of blood on paper (Figure 2) compare well with published spectra of hemoglobin in solution<sup>7</sup>. We hypothesized that the hemoglobin concentration was related to the difference in optical density between measurements made in the spectral region where hemoglobin absorbs and in the baseline region where it does not absorb.

**Red Blood Cell Lysis**

A diagnostic tool must give consistent results to be useful. We examined whether hemolysis affected the variability of light transmitted through paper spotted with blood. Figure 3a shows the cumulative percentage of samples with a given deviation from HemoCue for unlysed blood, mechanically lysed blood, and chemically lysed blood. Figure 3b shows the percent of samples for a given condition that are within  $\pm 1$  g/dL of the hemoglobin concentration obtained by HemoCue. The highest percentage of samples was within  $\pm 1$  g/dL when using mechanically lysed blood. On average, accuracy was slightly reduced when using chemically lysed blood. Accuracy was lowest when using unlysed blood. Though chemical lysis with sodium deoxycholate does not give the level of accuracy achieved with mechanical lysis, it represents an improvement over unlysed blood, and chemical lysis with pre-treated paper can be performed easily at the point of care.

We hypothesize that the free hemoglobin molecules in lysed blood spread more evenly throughout the paper than intact red blood cells do. This uniformity in spreading helps ensure that

light transmission through the square of paper measured by the spectrometer can be used to accurately determine the hemoglobin concentration for each blood sample.

### Wavelength and Drying Time Optimization

To be useful in a busy point-of-care setting, the test should not be overly dependent on the time between sample preparation and sample measurement.

To determine the appropriate wavelengths, absorption spectra from 450 to 800 nm of unlysed blood spotted on sodium deoxycholate-treated paper were measured versus time over the course of 30 min. for samples at four hemoglobin concentrations. Then, the difference in optical density between various pairs of wavelengths was plotted versus time, and the algorithm developed using the training set described in the section “Training and Validation with Patient Samples” was used to calculate the hemoglobin concentration. The algorithm was derived from training set spectra taken 2 min. after spotting; this algorithm was used to calculate hemoglobin concentration at each time point.

As shown in Figure 4, the absorbance of hemoglobin on paper was found to vary more with time at some wavelengths than at others. Some pairs of wavelengths give results that are stable over time, such as the difference between 528 nm and 656 nm (an average increase of 1.9 g/dL over 10 min., Figure 4a). Other pairs, such as 590 nm and 656 nm, result in increasing hemoglobin concentrations over time (an average of 4.9 g/dL over 10 min., Figure 4b). The choice of wavelength in the region where hemoglobin does not absorb had little effect on the results (data not shown). Other choices of wavelengths also give consistent results over time (such as 540 nm and 680 nm, with an average increase of 0.9 g/dL over 10 minutes), but 528 nm and 656 nm were chosen to match the availability of LEDs for our low-cost reader. The bandwidths of the LEDs (33 nm for the 528 nm LED and 25 nm for the 656 nm LED) include these other stable choices of wavelength.

Using 528 nm and 656 nm for calculation of hemoglobin concentration allows a healthcare worker to most accurately obtain a hemoglobin concentration without precisely monitoring the time from sample preparation to measurement, as long as the sample is measured within 10 min. For the remainder of the experiments, spectra were taken at 2 min. after spotting, and the absorbance difference between 528 nm and 656 nm was used to calculate hemoglobin concentration and compare to results determined by HemoCue.

### Volume Effects

Because the volume of blood in a finger-prick can vary, we examined how sensitive the method is to changes in blood spot volume. The absorbance difference between 528 nm and 656 nm was measured for various volumes of blood from three blood samples of different hemoglobin concentrations (Figure 5). The calculated hemoglobin concentrations for all volumes tested (5  $\mu$ L to 25  $\mu$ L) are not statistically different ( $p = 0.82$  for the high hemoglobin concentration,  $p = 0.37$  for the middle hemoglobin concentration, and  $p = 0.15$  for the low hemoglobin concentration), so this range of volumes is considered appropriate for measurements. Volumes of 2.5  $\mu$ L were also evaluated, but this volume of blood was not large enough to fill the 3 mm x 3 mm aperture used in the spectrometer and thus gave highly variable results.

To help health workers gauge that a sufficiently large volume has been spotted on the paper, eight small dots were laser printed onto the paper in the shape of a circle with diameter  $\sim 7.7$  mm. This circle holds approximately 10  $\mu$ L of blood. This circle also denotes where the sodium deoxycholate lysing agent has been dried onto the paper.

### Training and Validation with Hospital Patients



Results from the training and validation sets for both the spectrometer and the low-cost reader are shown in Figure 6. The Bland-Altman plots (Figure 6 c,d) show that the approach is accurate to within  $\pm 2$  g/dL of the HemoCue device for 98% (spectrometer) and 95% (low-cost reader) of samples. Our 95% limits of agreement are - 1.91 g/dL to + 2.10 g/dL for the spectrometer and - 2.07 g/dL to + 1.86 g/dL for the low-cost reader. It should be noted that none of the samples from hospitalized patients had a hemoglobin concentration higher than the normal range of hemoglobin concentrations for adult men (an upper bound of approximately 18.5 g/dL).

Gomez-Simon et al. tested the HemoCue against a laboratory hematology analyzer in a mobile blood donation setting and found 95% limits of agreement of - 0.68 g/dL to + 2.25 g/dL<sup>8</sup>. This point-of-care setting may be comparable to our targeted setting.

The WHO hemoglobin color scale also uses blood spotted on paper to determine hemoglobin concentration. With this method, the color of a blood spot is visually compared to reference standards ranging from 4 g/dL to 14 g/dL in gradations of 2 g/dL. This method is prone to inaccuracies: van den Broek et al. reported that the color scale was within 2 g/dL of a laboratory hemoglobin value in only 67% of cases<sup>9</sup>. Paddle, using one observer in ideal conditions, found the 95% limits of agreement for the color scale to be - 3.50 g/dL to + 3.11 g/dL<sup>5</sup>. The color scale strips are available in Malawi for ~\$0.02/test. We have summarized the reported accuracy and cost of these methods in Table 1. Our spectrophotometric method has the potential to provide increased accuracy over the current WHO color scale-based method with comparable or reduced cost. The accuracy of our method does not meet the  $\pm 7\%$  agreement limits set by CLIA.

While these results are encouraging, our study has a number of limitations. Our method does not give perfectly repeatable measurements for a given sample of blood. We hypothesize that these

inconsistencies arise due to a combination of variations in paper thickness and fiber arrangement or slight variations in how the blood was applied to the paper. We may be able to increase the accuracy in further studies by applying reagents to the paper to convert oxy- and deoxyhemoglobin to more chemically stable forms, such as the conversion to azide methemoglobin that is performed inside the HemoCue cuvettes. However, at present, the approach provides a more accurate, lower-cost method than is currently available with the WHO hemoglobin color scale and approaches the accuracy of the HemoCue in a mobile blood donation setting<sup>8</sup>. In this study, blood was applied with a micropipette and volume was controlled. A field test must be conducted where blood samples are collected directly from a fingerprick to evaluate the effects of a different method of application and of varying volumes of blood. In addition, the method has been validated with a relatively small sample size, which may not include patients with other hemoglobinopathies or blood disorders that could affect accuracy. Finally, while the HemoCue 201+ is approved for clinical use, it is not as accurate as laboratory reference methods based on spectrophotometry. The use of HemoCue as a reference standard may have biased estimates of the accuracy of the paper-based method described here. Further work is necessary to determine the impact of the potential sources of errors in the reference standard.

### **Cost Analysis and Biohazard Risk Reduction**

Whatman Chr4 chromatography paper, cut to 2.5 cm x 2.5 cm and combined with the necessary sodium deoxycholate, was calculated to have an approximately 99% decrease in cost when compared to HemoCue cuvettes. Pricing for HemoCue cuvettes is \$1.43/cuvette (as of January 2013) when purchased directly from HemoCue, Inc. in the United States and \$0.99/cuvette (as of January 2013), when purchased from MM African Technologies Ltd. in Malawi. In comparison, each 2.5 cm

x 2.5 cm strip of Whatman Chr4 costs \$0.006 when bought from Sigma-Aldrich. 10  $\mu$ L of 4% (w/v) sodium deoxycholate contains 0.4 mg of sodium deoxycholate, giving a per-test cost for the reagent of \$0.0003 when purchased from Sigma-Aldrich. Thus, the total material cost for the hemoglobin strip is \$0.0063, which is a 99.6% reduction in price from HemoCue cuvettes purchased in the United States and a 99.4% reduction from cuvettes purchased in Malawi. It should be noted these calculations represent only the material cost and do not include costs to manufacture or package the strips.

The plastic HemoCue cuvettes filled with blood necessitate considerations in minimizing biohazard risk. Our paper platform does not require sharps disposal, and it can be easily incinerated on-site to dispose of any potential biohazards.

The cost to build one prototype reader was approximately \$400 (see Table 2). The reusable plastic holder made for the low-cost device costs approximately \$4.00 to prototype on an in-house 3D printer. These calculations again only include the material cost for our device. There is significant potential to reduce this cost when the prototype is produced at higher volumes, particularly the cost of the fixture and the beamsplitter. We estimate that the materials cost of the device can be reduced by approximately one order of magnitude when produced at volumes of 10,000.

## Conclusions

This study demonstrates that chromatography paper is a low-cost medium that can be used for spectrophotometric detection of blood hemoglobin concentration with 98% (spectrometer) and 95% (low-cost reader) of samples within 2 g/dL of the reference. We developed a low-cost reader for the chromatography paper method for use in the field and validated the spectrometer and low-cost reader

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with clinical samples. The ability to reduce the per-test cost by ~99% and biohazard risk represents an important development in increasing the availability of a hemoglobin concentration assay in developing countries where anemia is prevalent. While performance must be improved to meet CLIA guidelines, current performance in the laboratory exceeds that reported for the WHO Hemoglobin Color Scale method in similar conditions. Moreover, the approach has the potential to significantly reduce the per-test cost of approaches that rely on more expensive plastic cuvettes. Field studies will be necessary to evaluate accuracy in relevant clinical settings and to establish whether the approach is cost-effective.

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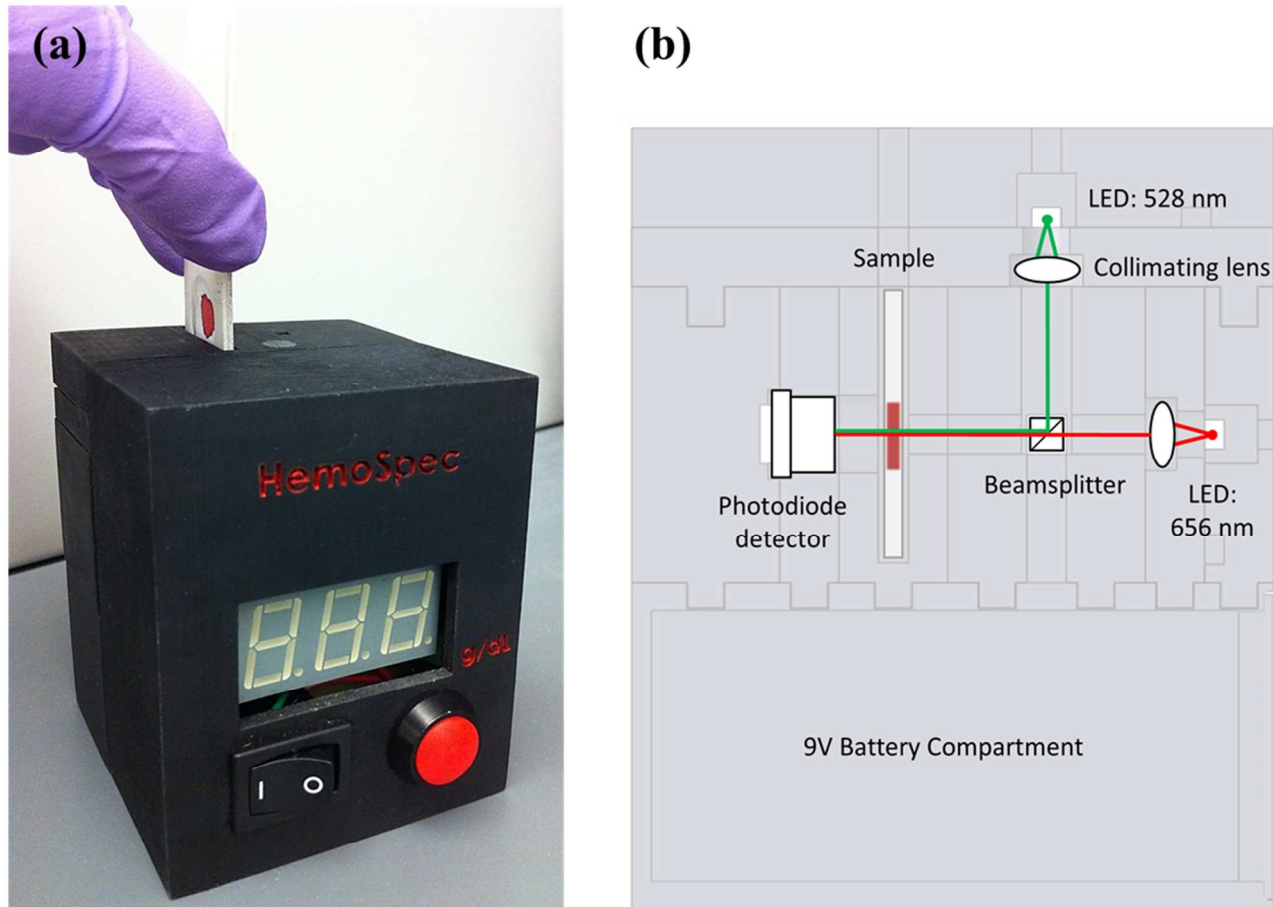
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**Table 1. Accuracy and Cost of Three Methods for Determining Hemoglobin Concentration**

Technique	Setting	Reported Accuracy (95% agreement limits unless otherwise stated)	Per-Test Cost	Reader Cost
HemoCue	Laboratory	- 0.5 to + 0.5 g/dL <sup>4</sup>	\$1.43 (US) \$0.99 (Malawi)	\$800 (US) \$470 (Malawi)
HemoCue	Field	- 0.68 to + 2.25 g/dL <sup>8</sup>	\$1.43 (US) \$0.99 (Malawi)	\$800 (US) \$470 (Malawi)
WHO Color Scale	Laboratory	- 3.50 to + 3.11 g/dL <sup>5</sup>	\$0.02 (Malawi)	\$8 (Malawi)
WHO Color Scale	Field	67% of measurements within 2 g/dL of reference <sup>9</sup>	\$0.02 (Malawi)	\$8 (Malawi)
Spectrophotometric measurement of blood spotted on chromatography paper	Laboratory	- 2.07 g/dL to +1.86 g/dL	\$0.006	\$400

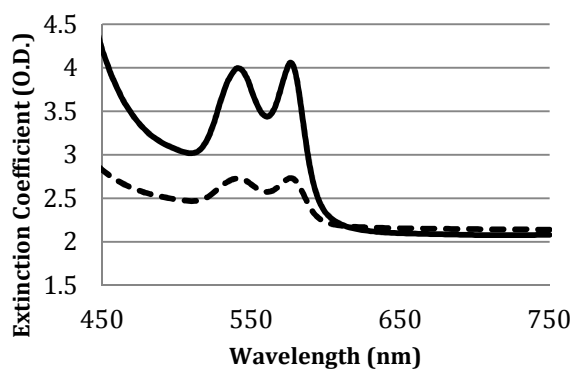
**Table 2. Prototype Material Costs for Low-Cost Reader**

	<b>Prototype Cost</b>	<b>Source/ Supplier</b>
Universal fixture	\$102.41	Materials cost using in-house 3D printer
2 high power LEDs	\$10.65	Mouser Electronics
2 plastic lenses	\$24.00	ThorLabs
Beamsplitter	\$143.00	ThorLabs
Photodiode/Amp	\$18.46	ThorLabs/ Mouser Electronics
Numerical display	\$2.10	Mouser Electronics
Signal processing	\$32.95	Mouser Electronics
Battery	\$8.75	Amazon.com
Switches	\$5.53	DigiKey
Printed Circuit Board	\$44.64	Advanced Circuits
Small Electronic Components	\$1.57	Mouser Electronics
<b>Total</b>	<b>\$394.06</b>	

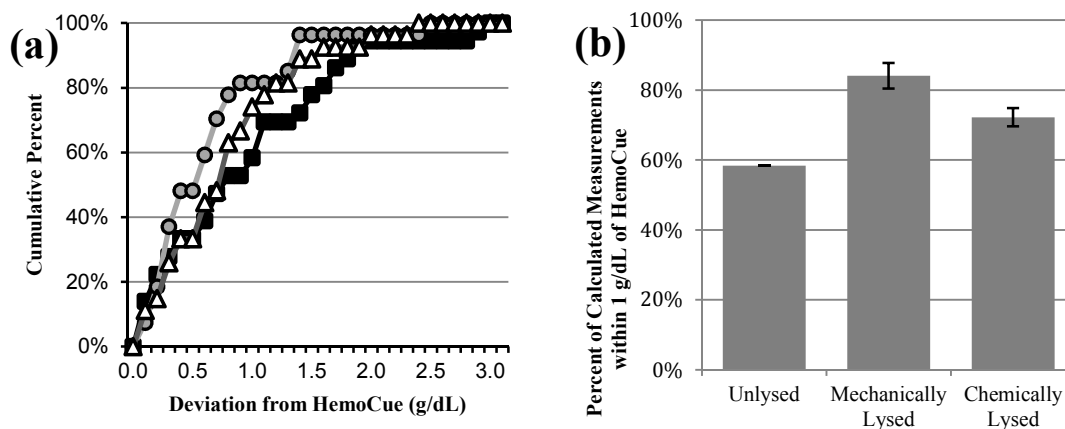


**Figure 1.** Photograph (a) and light path schematic (b) of self-contained low-cost reader.

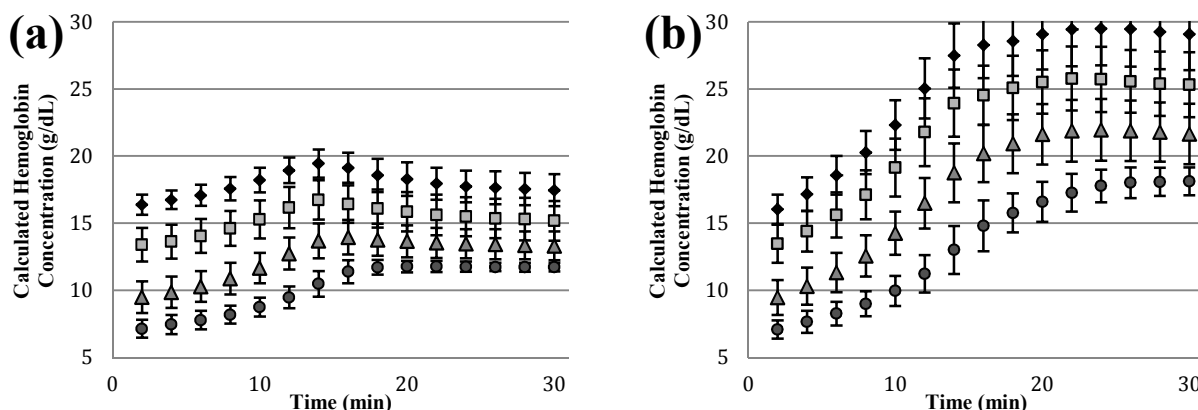




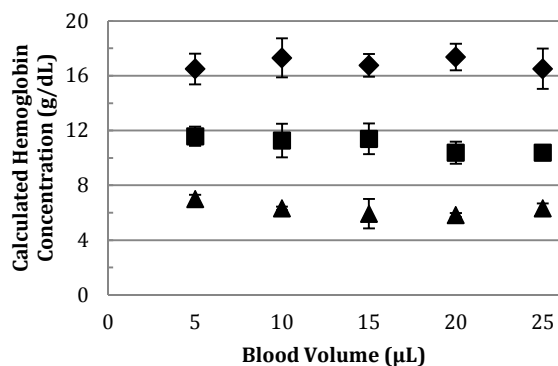
**Figure 2.** Extinction coefficient spectrum of unlysed blood on untreated Chr4 paper as calculated from collimated transmission measurement. Hemoglobin concentration as determined by HemoCue was  $14.9 \pm 0.3$  g/dL (solid line) and  $2.9 \pm 0.0$  g/dL (dashed line).



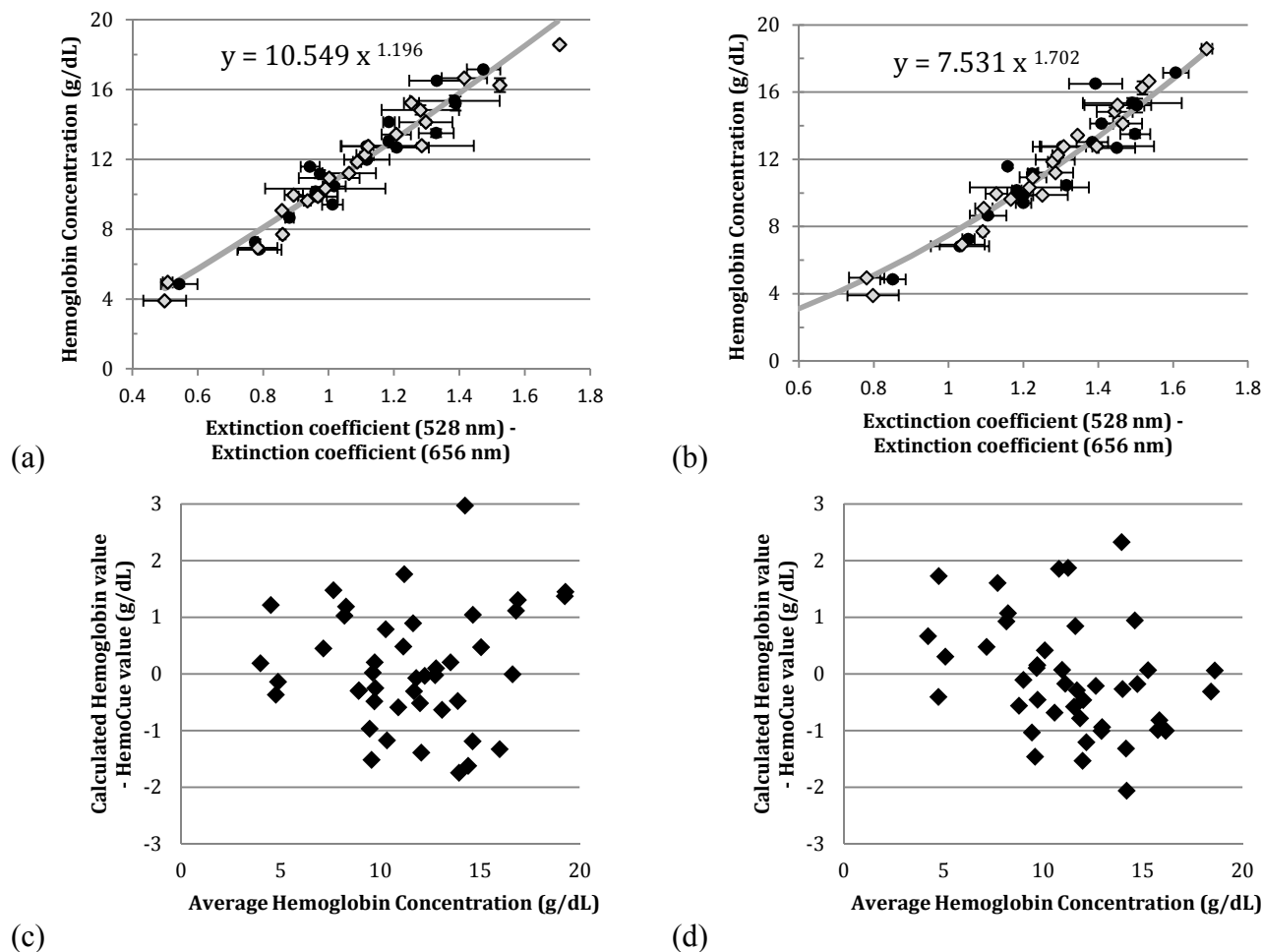
**Figure 3.** (a) Accuracy of spectral measurements compared to HemoCue measurements for unlysed blood on untreated paper (black squares,  $n = 36$ ), mechanically lysed blood on untreated paper (grey circles,  $n = 27$ ), and unlysed blood on sodium deoxycholate-treated paper (open triangles,  $n = 27$ ). The hemoglobin concentration of each sample was calculated using a training algorithm matching its condition (unlysed blood on untreated paper, mechanically lysed blood on untreated paper, or unlysed blood on sodium deoxycholate-treated paper). The concentrations investigated range from 2.4 g/dL to 16.1 g/dL. Deviations from HemoCue were binned in 0.1 g/dL increments. (b) The percentage of calculated hemoglobin measurements for each condition that were within  $\pm 1$  g/dL of HemoCue. Error bars represent one standard deviation of the measurements obtained from reversing the training and validation sets used to generate data shown in (a).



**Figure 4.** The difference between the extinction coefficients at 528 nm and 656 nm (a) gives reasonably constant calculated hemoglobin concentrations over the first 10 min after spotting (an average increase of 1.9 g/dL over 10 min), while the difference between, for example, 590 nm and 656 nm (b) gives increasing hemoglobin concentrations over time (an average increase of 4.9 g/dL over 10 min). Error bars represent one standard deviation for three measurements. Measurements were taken using unlysed blood from one donor, diluted with plasma and spotted on sodium deoxycholate-treated paper. Hemoglobin values are  $17.4 \pm 0.1$  g/dL (diamonds),  $13.3 \pm 0.1$  g/dL (squares),  $9.7 \pm 0.1$  g/dL (triangles), and  $6.4 \pm 0.1$  g/dL (circles) as determined with HemoCue.



**Figure 5.** Calculated hemoglobin concentration from three samples with different volumes of blood spotted on sodium deoxycholate-treated paper. All samples of a given hemoglobin concentration were from one blood donor. Hemoglobin concentration was calculated using the training set shown in Figure 6. Error bars represent one standard deviation for the average of 3 samples. HemoCue determined the concentration of the samples to be  $15.0 \pm 0.1$  g/dL,  $9.8 \pm 0.1$  g/dL, and  $5.3 \pm 0.1$  g/dL.



**Figure 6.** Training (solid circles) and validation (open diamonds) sets for the spectrometer (a) and the low-cost reader (b) using patient blood samples. Training,  $n = 44$ , validation,  $n = 42$ . Fit line and equation shown are for the training set. Hemoglobin concentration was determined by the average of four measurements on the HemoCue; vertical error bars represent one standard deviation of these measurements. Horizontal error bars represent one standard deviation of two replicate measurements on the spectrometer (a) and low cost reader (b). Bland-Altman plots comparing the HemoCue to the hemoglobin values calculated for the validation set by the spectrometer (c) and the low-cost reader (d).

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