Inexpensive Urinalysis Test Strips to Screen for Diabetes in Developing Countries

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***Abstract* - Sub-Saharan Africa is increasingly experiencing the double burden of communicable and noncommunicable diseases. Many of these diseases, including diabetes, remain prevalent despite the availability of viable treatment options. Lack of access to screening tools frequently prevents individuals from seeking a diagnosis or pursuing treatment. The development of alternative screening devices in the form of low cost test strips has the potential to surmount existing barriers to treatment, allowing earlier intervention in the progression of diabetes. This article presents a demonstration that modified published protocols for glucose and ketone detection in solution - two of the principal chemical signatures of diabetes - are reproducible on test strips manufactured using composite rubber-foam stamps to print chemical reagents on filter paper. These test strips contain specific chemicals which, in the presence of disease markers, cause a visible color change and defined intensity gradient that can be experimentally verified. The creation of an affordable and effective urinalysis test strip has the ability to make screening for disease more accessible in developing nations.**

Keywords – global health, diabetes; screening test; point-of-care, low-cost diagnostics, ketone test; glucose test

I. Introduction

Noncommunicable diseases, once considered endemic solely to industrialized nations, are increasingly affecting developing nations as well. In Sub-Saharan African countries like Sierra Leone, noncommunicable diseases account for 26% of all deaths; by 2030, this number is expected to surpass 50% [1][2]. One particularly dangerous noncommunicable disease is diabetes. Worldwide, over four hundred million adults are currently living with diabetes [3], with three quarters of this population living in developing nations [4]. Additionally, communicable diseases like malaria, AIDS, tuberculosis and dengue continue to be uniquely prominent in developing nations, leading to a double burden of communicable and noncommunicable diseases. Addressing a lack of accessibility and affordability are two of the most important components of combating the increasing prevalence of both communicable and noncommunicable diseases.

To counter systemic limitations of poorly funded healthcare systems, Sierra Leone, along with many other developing nations, relies on the use of a civic healthcare structure utilizing community health workers (CHWs). CHWs are community members who serve as connectors between healthcare providers and patients in community settings [5]. Entrusted by their peers to communicate with outside health officials, CHWs are often the most effective distribution pathway of health information and services in rural as well as urban areas. By providing CHWs with affordable ways to screen patients for communicable and noncommunicable diseases, individuals with diseases like diabetes can be afforded earlier intervention in the disease process. Development of effective and inexpensive preventative disease screening methods for use in the developing world has thus become more important than ever [6].

Currently, blood testing and, less often, urinalysis are used to assess diabetes. When testing for diabetes, patients can be screened to detect disease markers, then diagnosed to establish disease presence. For diabetes screening purposes, a method for locally manufacturing two-parameter versions of urinalysis test strips can be adopted for the purpose of screening for diabetes. In this method, detection reagents are hand-stamped via foam stamps onto filter paper and then air-dried [7]. This stamping system has the ability to grant a currently inaccessible audience knowledge of their health status. Comparable urinalysis test strips (URS-2K) screen for glucose at 5, 15, 30, 60, and 110 mmol/L and for ketones at 0.5, 1.5, 4.0, 8.0, and 16 mmol/L. These strips demonstrate comparable sensitivity and specificity to commercially available urinalysis test strips, with a color change and gradient verified through ImageJ analysis. Such strips are also affordable, simple enough to be interpreted at home, and can be locally manufactured and easily distributed in Sub-Saharan Africa. In this paper, we demonstrate that a hand-stamped method of manufacture can be used to produce urinalysis test strips capable of detecting two diabetes markers, glucose and ketones.

II. Screening Methods

Worldwide, a plethora of technologies are available for the purpose of testing for diabetes; not all of these options, however, are suitable for use in developing nations. Internationally, the most common method of testing for diabetes is blood analysis; blood assays can be performed as

an analysis of fasting glucose, random glucose, or HbA1C. Blood tests are extremely sensitive and specific, making them ideal for diagnosing diabetes. Because of their inherent invasive nature and associated risk of blood contact, however, blood tests require a trained individual to perform them. Such caveats reduce the efficiency of blood analysis, but blood tests are often the only testing method for which medical centers in developing areas can currently obtain supplies. Blood tests, however, are also expensive and much of the general public is forced to forego being tested for diabetes because of cost.

Within diabetes testing, technologies are typically only employed to provide a confirmation of diabetes diagnosis. To bridge the gap between diabetes testing and the general public, an inexpensive screening device can be introduced that detects diabetes markers. Screening patients offers an intermediate opportunity to separate healthy individuals from those that may have diabetes. Through screening, more accessible testing methods can be introduced to the community, reducing the need for expensive blood tests and testing a larger population than blood tests could.

Urinalysis test strips provide an alternative method of diabetes testing that is well-suited for screening purposes. These strips are simple in principle as well as in use: they change color when exposed to a solution containing a marker for disease. Urinalysis screening for diabetes is fairly common worldwide and typically requires testing for glucose and ketones. If the urine of a patient tests positive for these markers, the likelihood of diabetes as the cause is significant, and the patient should be advised to seek further medical attention. Ten-parameter urinalysis test strips that screen for glucose, ketones, bilirubin, protein, specific gravity, leukocytes, blood, urobilinogen, nitrates, and pH are currently available in industrialized countries, as well as countries in the developing world. These strips, however, are not easily accessible to the general public, especially in rural areas, and can be expensive and difficult to interpret without medical knowledge. Two parameter versions of urinalysis test strips are also available for purchase, but these strips are difficult to source, making them relatively expensive for the average individual in a developing nation to purchase.

Used only in rural settings, a third, simpler method of screening and, occasionally, diagnosing relies on the presence of ants to determine glucose concentration in urine. To screen for diabetes, individuals are instructed to urinate on the ground; if, after a certain amount of time, ants are attracted to the urine, the individual may have diabetes. This method is not as reliable as other methods of screening because of its ambiguity and dependence on environment. Screening via ants, however, is virtually free and does not require extensive medical knowledge that the general public does not possess. Still, when testing for diabetes via this method, results are inconsistent and can easily spawn misdiagnoses.

Research into additional methods of diabetes screening specifically for developing nations is currently taking place. Creative approaches using an algorithm associating handgrip strength, weight, blood pressure, and BMI with the likelihood of having diabetes [8]; inkjet-printed blood plasma glucose strips and urinalysis test strips [9][10]; paper-based microfluidic strips integrated with telemedicine systems [11]; reusable nanoparticle based sensors; light sensitive devices [12]; and tools detecting the presence of a diabetes marker in fingernails are all currently being studied [13]. These technologies, however, are still in development and are not currently being utilized in low resource settings.

The proposed hand-stamped urinalysis test strip has proven to perform competitively alongside current diabetes testing methods. By synthesizing known urinalysis procedures and protocol with simple methods of manufacture [7], a suitable screening device can be developed and implemented with limited capital. The simple screening process allows for use of a two-parameter hand-stamped diabetes urinalysis device in remote, rural communities, making diabetes screening accessible and satisfying the demand for additional methods of diabetes testing.

III. Methodology

A. Glucose Detection Assay

The established Sigma Aldrich protocol using glucose oxidase (GOD) to produce a detectable color change in the presence of glucose was used as the foundation for all the experiments regarding detection of glucose performed in this paper [14]. The general chemical reaction that produces this color change is as follows:

$$\text{glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O \quad (1)$$

$$H_2O_2 + \text{chromogen} \rightarrow \text{color change} \quad (2)$$

Following analysis of enzyme concentration, chromogen selection, and chromogen concentration, the Sigma Aldrich protocol was adapted to best fit the intended application of hand-stamped urinalysis screening devices. Each concentration or other selection was determined by an individual assay; assays were prepared and completed according to the following methods.

B. Sigma-Aldrich Glucose Detection Reagent Preparation Methods

1) 50 mM Sodium Acetate Buffer: A solution of 50 mL purified water and 340 mg sodium acetate trihydrate (Sigma-Aldrich Product Number S8625) was prepared. Orthophosphoric acid was used to adjust to pH ~5.

2) 0.21 mM o-Dianisidine Solution: 5 mg o-dianisidine dihydrochloride (Fisher Scientific Product Number AAA1717503) was combined with 2 mL purified water in a vial protected from light. From this solution, 1.34 mL of o-

dianisidine solution was diluted to 50 mL using sodium acetate buffer.

3) Glucose Oxidase Enzyme Solution: In 1 mL cold sodium acetate solution, 0.1 mg glucose oxidase (Sigma-Aldrich Product Number G2133) was dissolved. From this solution, 0.1 mL were further diluted to 5 mL with sodium acetate buffer.

4) Horseradish Peroxidase Enzyme Solution: A solution of 0.4 mg of horseradish peroxidase (Sigma-Aldrich Product Number P8250) in 1 mL purified water was prepared.

5) In-tube Reagent Solution: 0.1 mL of the diluted glucose oxidase enzyme solution, 0.1 mL of the horseradish peroxidase enzyme solution, and 2.4 mL of diluted o-dianisidine/buffer solution were combined in a test tube. In conjunction, a second test tube was prepared following the same procedure to serve as a control.

6) Verification Method: 0.5 mL of 10% w/v glucose solution was added to the glucose detection reagent solution to trigger color change and was compared to the control vial to which 0.5 mL of distilled water was added.

C. Glucose Detection Reagent Preparation Methods for Enzyme Selection

1) 50 mM Sodium Acetate Buffer: Buffer solution was prepared according to methods discussed in III.B.1.

2) 0.21 mM o-Dianisidine Solution: Solution was prepared according to methods discussed in III.B.2.

3) Glucose Oxidase Enzyme Solutions: Four solutions with different enzyme concentration were prepared. In separate 1 mL cold sodium acetate solution aliquots, 0.1, 0.3, 0.5, 0.8 mg glucose oxidase (Sigma-Aldrich Product Number G2133) were dissolved. From each one of these solutions, 0.1 mL was further diluted to 5 mL with sodium acetate buffer.

4) Horseradish Peroxidase Enzyme Solutions: Four solutions of 0.4, 0.6, 0.8, 1 mg of horseradish peroxidase (Sigma-Aldrich Product Number P8250) in separate 1 mL purified water aliquots were prepared.

5) In-tube Reagent Solution: Sixteen tubes were utilized to compare different combinations of enzymes. In each tube, 0.1 mL of horseradish peroxidase solution and 0.1 mL of glucose oxidase solution were mixed with 2.4 mL of o-dianisidine and buffer solution so that individual solutions existed of concentrations of all possible combinations of enzymes using 0.1, 0.3, 0.5, and 0.8 mg glucose oxidase per 1 mL cold buffer solution before dilution and 0.4, 0.6, 0.8, and 1.0 mg horseradish peroxidase per 1 mL cold water.

6) Verification Method: 0.5 mL of 10% w/v glucose solution was added to each one of the 16 different enzyme cocktails to trigger color change. The solutions were then dropped onto filter paper and allowed to dry to preserve the results for future record.

D. Glucose Detection Reagent Preparation Methods for Chromogen Concentration Selection

1) 50 mM Sodium Acetate Buffer: Buffer solution was prepared according to methods discussed in II.B.1.

2) o-Dianisidine Solutions: 5 mg o-dianisidine dihydrochloride (Fisher Scientific Product Number AAA1717503) was combined with 2 mL purified water in a vial protected from light. From this initial o-dianisidine solution, five batches of chromogen solution of varying concentrations were created by diluting the initial solution with quantities of sodium acetate buffer as specified in Table 3.

TABLE 1. METHOD OF PREPARATION OF FIVE DIFFERENT CHROMOGEN CONCENTRATION SOLUTIONS. RESULTING SOLUTIONS WERE 0X, 1X, 2X, 4X, AND 8X THAT OF THE ORIGINAL DILUTED O-DIANISIDINE SOLUTION SPECIFIED IN THE SIGMA-ALDRICH PROTOCOL.

chromogen concentration	mL of o-dianisidine solution	mL of sodium acetate buffer
0x	0	5
1x	0.134	5
2x	0.134	2.5
4x	0.134	1.25
8x	0.134	0.75

3) Glucose Oxidase Enzyme Solution: In 1 mL cold sodium acetate solution, 0.1 mg glucose oxidase (Sigma-Aldrich Product Number G2133) was dissolved. From this solution, 0.1 mL were further diluted to 5 mL with sodium acetate buffer.

4) Horseradish Peroxidase Enzyme Solution: A solution of 2.0 mg of horseradish peroxidase (Sigma-Aldrich Product Number P8250) in 2.5 mL purified water was prepared.

5) Stamping Method: To stamp the solutions, 0.1 mL of glucose oxidase enzyme solution and 0.1 mL horseradish peroxidase enzyme solution were combined with 2.4 mL of diluted o-dianisidine solution with buffer and stamped onto filter paper via a foam stamp. Concentrations of o-dianisidine solution were varied so that five separate stamping solutions were created using 0x, 1x, 2x, 4x, and 8x concentration solutions, respectively. The paper was then allowed to dry.

6) Verification Method: Once strips were allowed to dry, glucose solutions of 0, 5, 15, 30, 60, and 110 mmol/L were each applied to separate strips to induce color change and allow observation of chromogen effectiveness with varying glucose concentration.

E. Glucose Detection Reagent Preparation Methods for Chromogen and Enzyme Concentration Selection

1) 50 mM Sodium Acetate Buffer: Buffer solution was prepared according to methods discussed in III.B.1.

2) o-Dianisidine Solutions: Quantities of o-dianisidine measuring 0, 5, 10, and 20 mg were individually combined with 2 mL purified water in separate vials protected from light. From these solutions, 0.134 mL of each chromogen-containing solution was diluted to 5 mL using sodium acetate buffer.

3) Glucose Oxidase Enzyme Solutions: Five solutions with different enzyme concentration were prepared. In separate 1 mL cold sodium acetate solution aliquots, 0.0, 0.1, 0.3, 0.5, 0.8 mg glucose oxidase (Sigma-Aldrich Product Number G2133) were dissolved. From each one of these solutions, 0.1 mL was further diluted to 5 mL with sodium acetate buffer.

4) Horseradish Peroxidase Enzyme Solutions: Five solutions of 0, 0.4, 0.6, 0.8, 1 mg of horseradish peroxidase (Sigma-Aldrich Product Number P8250) in separate 1 mL purified water aliquots were created.

5) Stamping Method: One hundred filter paper strips were utilized to compare different combinations of enzymes. Stamping solutions were created by combining 0.1 mL of horseradish peroxidase solution and 0.1 mL of glucose oxidase solution with 2.4 mL of o-dianisidine and buffer solution so that individual solutions existed of enzyme concentrations of all possible combinations of enzymes using 0.0, 0.1, 0.3, 0.5, and 0.8 mg glucose oxidase per 1 mL cold buffer solution before dilution and 0.0, 0.4, 0.6, 0.8, and 1.0 mg horseradish peroxidase per 1 mL cold water.

6) Verification Method: Once strips were allowed to dry, a glucose solution of 10% w/v was applied to the strips, and any screening strip color changes were observed.

F. Ketone Detection Assay

The assay for the detection of ketones performed as part of this research paper was retrieved from US patent 4147514 issued in 1979 [15]. The general chemical reaction that produces this color change is as follows:

$$\text{acetoacetic acid} + \text{sodium nitroprusside} \rightarrow \text{color change} \quad (3)$$

After pH and chromogen concentration analysis, the protocol developed by Mager et al. was adapted to best fit the intended application of hand-stamped urinalysis screening devices. To obtain acetoacetic acid, a ketone, in order to test the screening ketone strips, ethyl acetoacetate was reacted for 48 hours at room temperature with 1 N KOH to yield the product via ester hydrolysis.

G. Completion of In-tube Testing for the Development of Ketone Assay

1) Ketone Detection Solution: To 250 mg sodium nitroprusside (VWR Product Number IC15206125), 5 mL of purified water were added. In a second tube, 2.5 g magnesium sulfate heptahydrate (Fisher Scientific Product Number M63500) were also mixed to obtain a saturated solution. A 1 M KOH solution was used to adjust the pH to ~9.5.

2) In-tube Method: For the completion of the reaction, 0.1 mL of solution was placed in each of two test tubes.

3) Verification Method: The ketone acetoacetic acid solution at concentration 50 mg/mL was added to the ketone detection solution at a 1:1 ratio. This solution was compared to a control tube in which the ketone detecting agent was mixed with distilled water at a 1:1 ratio.

H. Comparison of Ketone Detection Reagent Preparation Methods with and without Glycine

1) Ketone Detection Solution 1: To 175 mg sodium nitroprusside (VWR Product Number IC15206125), 2.5 mL of distilled water were added and, in a second tube, 1.75 g magnesium sulfate heptahydrate (Fisher Scientific Product Number M63500) was diluted in solution. A 1 M KOH solution was used to adjust the pH to ~9.5.

2) Ketone Detection Solution 2: 175 mg crystallized sodium nitroprusside (VWR Product Number IC15206125) was diluted to 2.5 mL with distilled water, and 1.75 g magnesium sulfate heptahydrate (Fisher Scientific Product Number M63500) were added. In addition, 0.34 mg of glycine (Sigma Aldrich product G7126) was added. A 1 M KOH solution was used to adjust the pH to ~7.5.

3) Stamping Method: To create screening strips, both the ketone detection solutions were stamped onto filter paper via a foam stamp. The paper was then allowed to dry.

4) Verification Method: The solution of acetoacetic acid was diluted in distilled water to concentration of 0, 0.5, 1.5, 4, 8, 16 mmol/L. Once strips were allowed to dry, the ketone acetoacetic acid solutions were applied to the strips and screening strip color changes were observed.

I. Finalized Glucose and Ketone Detection Reagents Preparation Methods and Sensitivity Test for 500 Test Strips

1) Glucose

a) 50 mM Sodium Acetate Buffer: Buffer solution was prepared according to methods discussed in III.B.1.

b) 0.84 mM o-Dianisidine Solution: For this solution, 20 mg o-dianisidine dihydrochloride (Fisher Scientific Product Number AAA1717503) was combined with 2 mL purified water in a vial protected from light. From this solution, 1.6 mL of o-dianisidine solution was diluted to 60 mL using sodium acetate buffer.

c) Glucose Oxidase Enzyme Solution: In 1 mL cold sodium acetate solution, 0.5 mg glucose oxidase (Sigma-Aldrich Product Number G2133) was dissolved. From this solution, 0.1 mL were further diluted to 5 mL with sodium acetate buffer.

d) Horseradish Peroxidase Enzyme Solution: A solution of 1 mg of horseradish peroxidase (Sigma-Aldrich Product Number P8250) in 2.5 mL purified water was created.

e) Stamping Method: To stamp the solutions, 2.5 mL of solutions glucose oxidase enzyme solution and 2.5 mL horseradish peroxidase enzyme solution were combined with 60 mL of o-dianisidine solution and stamped onto filter paper via a foam stamp. The paper was then allowed to dry.

f) Verification Method: Once strips were allowed to dry, glucose solutions of 0, 5, 15, 30, 60, and 110 mmol/L, were applied to the strips and any screening strip color changes were observed.

2) Ketones

a) Ketone Detection Solution: To 65 mL of purified water, 3.25 g sodium nitroprusside (VWR Product Number IC15206125) and 32.5 g magnesium sulfate heptahydrate (Fisher Scientific Product Number M63500) were added. A 1M KOH solution was used to adjust the pH to ~9.5.

b) Stamping Method: Screening strips were stamped according to III. I. 3.

c) Verification Method: Verification was executed according to methods III.I.4.

IV. Results

A. Sigma-Aldrich Glucose Detection

The initial Sigma-Aldrich protocol was completed in a test tube to validate the chemistry (i.e., a color change must occur). After reactions were allowed to proceed, color change occurred as visible in Fig. 1. For additional experiments, enzyme and chromogen concentrations were adjusted to maximize reaction efficiency for urinalysis; additional chromogens were also studied by directly substituting potassium iodide and combinations of potassium iodide and o-dianisidine for o-dianisidine. This chromogen selection study, however, proved o-dianisidine to be the most effective chromogen and additional chromogen research was not necessary.

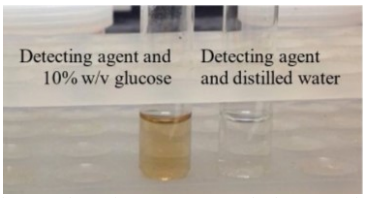

Fig. 1: Samples of glucose detection reagents and glucose solution. **Left:** Glucose solution was combined with enzyme and chromogen solutions to elicit a color change. **Right:** A control was created substituting water for glucose solution.

B. Enzyme Selection for Glucose Detection

To optimize reactions for the purpose of diabetes screening, a study was performed focusing on enzyme concentration selection. For this study, varying concentrations of glucose oxidase and horseradish peroxidase were used to perform an assay; the varying concentrations resulted in a color change gradient as visible in Fig. 2.

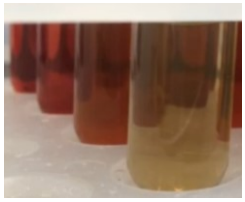

Fig. 2: Observable color change of the solution when the glucose detecting agents were mixed with 10% w/v glucose solution. The intensity of the color is varied due to the variation in concentration of horseradish peroxidase and glucose oxidase enzymes in solution. Enzyme concentration decreases from left to right.

From these results, it was concluded that a combination of 0.1 mg glucose oxidase in 1 mL cold sodium acetate buffer and 0.8 mg horseradish peroxidase in 1 mL of cold water would yield the most effective results. This was concluded after noting the color change of the aforementioned enzyme combination to be the most visible when compared to the other combinations after an extended period of time. It was also noted that after color changes proceeded, reagent combinations using more enzyme than 0.1 mg glucose oxidase per 1 mL buffer and 0.8 mg horseradish peroxidase per 1 mL cold water did not demonstrate a significant color gradient or additional benefits in comparison to the selected enzyme combination.

C. Chromogen Concentration Selection for Glucose Detection

To identify if adjusting the quantity of chromogen in the reagent solutions would have an effect on the intensity of the color change after combining reagents with glucose, a chromogen concentration selection study was completed. After comparing reactions utilizing chromogen concentrations 0x, 1x, 2x, 4x, and 8x that of the original Sigma-Aldrich, results pictured in Fig. 3 were achieved.

From the portrayed results, it was found that pursuing a chromogen concentration 8x that of the original would result in a loss of accuracy in glucose concentration detection and would not be considered as an option in a final concentration scenario. In addition, the 8x concentration did not provide significant advantages over 1x, 2x, and 4x concentrations. Lower concentration values from 1x through 4x resulted in a sensitive color gradient; because all of these concentrations were visible, these same lower values were also used in additional testing to determine if enzyme concentration in combination with chromogen concentration would also have an effect on color change intensity.

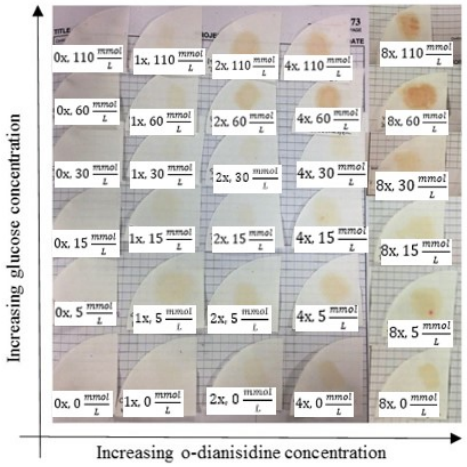

Fig. 3: From left to right, glucose detecting solution containing 0x, 1x, 2x, 4x, 8x o-dianisidine. From bottom to top, detecting reagents reacted with 0, 5, 15, 30, 60, and 110 mmol/L glucose solutions respectively.

D. Chromogen and Enzyme Concentration Selection for Glucose Detection

To select a final chromogen and enzyme concentration combination, an experiment was completed using varying concentrations of glucose oxidase, horseradish peroxidase, and o-dianisidine solutions. From these results, pictured in Fig. 4, a final reagent combination utilizing 0.5 mg glucose oxidase in 1 mL of cold buffer then further diluted, 0.4 mg horseradish peroxidase in 1 mL of cold purified water, and 20 mg o-dianisidine in 2 mL buffer and further diluted was determined.

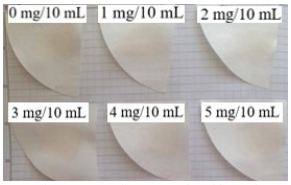

Fig. 4. Test strips featuring reaction cocktails of enzyme concentrations with chromogen concentration 4x that of the original Sigma-Aldrich protocol. Sample represents increasing glucose oxidase concentration in mg/mL H2O.

Chromogen concentration had the most significant effect on color change intensity, with the 4x o-dianisidine concentration producing the most visible color change in comparison to lower concentrations. Enzyme concentrations also produced visible gradients for lower enzyme concentrations, but concentrations greater than 0.5 mg glucose oxidase in 1 mL buffer and 0.4 mg horseradish peroxidase in 1 mL water did not provide significant advantage over the enzyme concentration combination selected as most effective.

E. Mager Ketone Detection

The aim of this test was to obtain visible color change when reacting an acetoacetic acid solution with the ketone detecting agent. For the previously described protocol, a deep brown color was observable in the solution containing acetoacetic acid solution. This solution was compared to a control tube in which the color of the test was of a lighter shade, due to reagent coloring. This pattern is observable in Fig. 5. From this experiment, the use of sodium nitroprusside for the detection of ketones proved to be the most reliable indicator. For this reason, the following protocols focused on the use of the selected indicator, which is also utilized by commercialized test strips such as URS-2K.

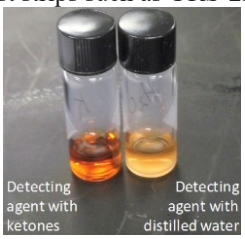

Fig. 5: Test tubes containing 100 µL of ketone detection assay each. **Left:** 100 µL of acetoacetic acid at a concentration of 50 mg/mL was added to the ketone detecting solution. **Right:** distilled water was added at a 1:1 ratio to the ketone detection reagents.

F. Impact of Glycine for Ketone Detection

The original Mager patent suggests that the addition of an amino acid, such as glycine, demonstrated unexpected, beneficial properties during testing.14 To inspect these effects, reagents solutions with and without glycine were compared. Test strips manufactured using the specified reagent combination successfully detected the presence of ketones in solution at varying concentrations of 0, 0.5, 1.5, 4, 8, 16 mmol/L as observable in Fig. 6. After observation of the results, it was possible to conclude that glycine had no visible effect on color change and would not be pursued as a reagent in the future. This lack of difference in color change could, however, be attributed to the use of simulated ketone solution as opposed to urine samples when verifying the reaction.

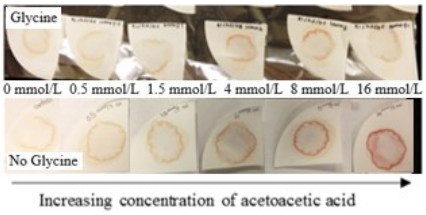

Fig. 6: Test strips stamped with a ketone detection assay using a foam stamp onto filter paper were dried at room temperature (20°C). Solutions of acetoacetic acid at a concentration of 0, 0.5, 1.5, 4, 8, 16 mmol/L were dropped on the ketone detecting test strips. **Top:** The ketone detecting solution containing glycine. **Bottom:** The ketone detection reagent which does not contain glycine.

G. Finalized Glucose and Ketone Detection Sensitivity Test

Test strips manufactured using the two protocols described in Section II successfully detected the presence of glucose and ketones in solution at varying concentrations. Commercially available test strips detect glucose in solution at concentrations of 0, 5, 15, 30, 60, and 110 mmol/L, and

ketones in solution at concentrations of 0, 0.5, 1.5, 4, 8, and 16 mmol/L. Test strips were exposed to solutions comprising glucose and ketones dissolved in distilled water at these concentrations. The correlation of strip color gradient after testing to glucose and ketone concentration gradient is visible in Fig. 7.

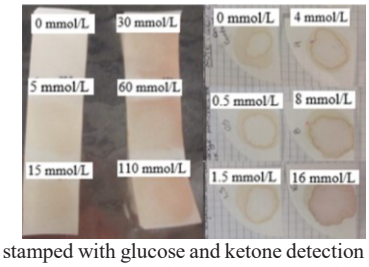

Fig. 7: Test strips stamped with glucose and ketone detection assays display comparable sensitivity to commercial test strips. **Left:** Filter paper stamped with glucose detection reagents, after the addition of solutions of glucose in distilled water, with varying glucose concentrations in mmol/L. **Right:** Filter paper stamped with ketone detection reagents, after the addition of solutions of acetoacetic acid in distilled water, with varying acetoacetic acid concentrations in mmol/L.

H. ImageJ Analysis of Glucose and Ketone Sensitivity Assays

To validate the resulting color gradient from glucose and ketone sensitivity assays, RGB Histogram and gray value analysis were completed using ImageJ to obtain average red, green, and blue values and respective standard deviations, average gray value, min gray value, and max gray value for the assays.

1) Glucose

For glucose sensitivity analysis, RGB histogram values are visible in Table 2 and gray value analysis in Table 3. For the glucose color change reaction, color changes from a light brown at lower glucose concentrations to a deeper reddish-brown at higher glucose concentrations.

TABLE 2. RGB HISTOGRAM ANALYSIS INCLUDING AVERAGE RED, BLUE, GREEN VALUES AND STANDARD DEVIATIONS FOR EACH AT 0, 5, 15, 30 ,60, AND 110 MMOL/L OF GLUCOSE.

Glucose mmol/L		Sample size	Red	Green	Blue
0	μ	36	222.5	216.1	205.1
	σ		1.5	1.7	2.0
5	μ	36	217.2	208.0	196.3
	σ		1.8	2.2	2.4
15	μ	36	215.6	205.0	193.0
	σ		1.7	1.8	2.2
30	μ	36	183.2	171.4	157.9
	σ		2.1	2.5	2.8
60	μ	36	177.5	165.2	153.5
	σ		2.0	2.3	2.7
110	μ	36	156.3	144.2	130.4
	σ		2.4	2.6	2.8

As glucose concentration increases, these colors also increase in intensity. These trends are quantified via RGB Histogram and gray value analysis; values corresponding to 0 mmol/L decrease through 110 mmol/L. This trend verifies existence of a gradual color and intensity gradient correlating with glucose solution concentration, validating experimental results and demonstrating the ability of hand-stamped urinalysis screening strips for diabetes to perform with results comparable to OEM strips.

TABLE 3. GRAY VALUE ANALYSIS INCLUDING MEAN, MIN, AND MAX FOR 0, 5, 15, 30, 60, AND 110 MMOL/L OF GLUCOSE.

mmol/L	Sample size, N	Mean gray value	Standard Deviation, σ	Min gray value	Max gray value
0	36	214.5	1.6	210.3	218.8
5	36	206.9	2.0	201.2	213.6
15	36	204.4	1.8	199.0	209.4
30	36	170.6	2.3	163.4	177.2
60	36	165.3	2.2	158.1	171.2
110	36	143.4	2.5	135.9	150.6

2) Ketones

For ketones sensitivity analysis, RGB histogram values are visible in Table 4 and gray value analysis in Table 5. For the ketones color change reaction, color varies from brown at lower ketone concentrations to purple at higher ketone concentrations.

TABLE 4. RGB HISTOGRAM ANALYSIS INCLUDING AVERAGE RED, BLUE, GREEN VALUES AND STANDARD DEVIATIONS FOR EACH AT 0, 0.5, 1.5, 4, 8, AND 16 MMOL/L OF KETONES.

Ketones mmol/L		Sample size, N	Red	Green	Blue
0	μ	36	183.5	178.7	173.5
	σ		3.4	3.4	3.7
0.5	μ	36	179.6	172.9	167.5
	σ		3.3	3.3	3.6
1.5	μ	36	176.0	166.8	162.8
	σ		3.3	3.8	4.2
4	μ	36	184.5	167.2	165.7
	σ		3.7	5.7	5.6
8	μ	36	174.5	147.8	149.8
	σ		3.1	4.7	4.4
16	μ	36	157.9	118.2	122.0
	σ		3.7	5.0	4.7

TABLE 5. GRAY VALUE ANALYSIS INCLUDING MEAN, MIN, AND MAX FOR 0, 0.5, 1.5, 4, 8, AND 16 MMOL/L OF KETONES.

mmol/L	Sample size, N	Mean gray value	Standard Deviation, σ	Min gray value	Max gray value
0	36	178.6	3.4	166.9	190.1
0.5	36	173.3	3.3	162.1	184.7
1.5	36	168.5	3.6	155.8	180.9
4	36	172.5	4.8	156.1	186.7
8	36	157.3	3.9	143.5	170.1
16	36	133.6	4.3	118.4	148.0

These colors also increase in intensity as ketone concentration increases. Color change from brown to purple occurs from 1.5 to 4 mmol/L. These trends are visible via both RGB histogram and gray value analysis; values corresponding to 0 to 1.5 mmol/L decrease until 4 mmol/L, then decrease again from 4 to 16 mmol/L. These trends

signify a gradual color gradient and significant color change from 1.5 to 4 mmol/L, validating experimental results and signifying that hand-stamped urinalysis screening strips for diabetes can perform at a level comparable to that of similar OEM strips.

V. DISCUSSION

The primary objective of this study was to demonstrate that a novel stamping system could be used to print screening test strips. In this study, the test strips in question were printed with biochemical assays capable of detecting ketones and glucose in solution – two markers of diabetes. Future studies will seek to print test strips with assays capable of detecting a multitude of chemical markers of disease and other various conditions. Successfully expanding the application of our stamping system for screening test strips would significantly improve their viability as products in the developing world.

The secondary objective of this study was to print test strips capable of detecting biochemical markers with similar sensitivity to commercially available test strips. Tables 2 through 5 demonstrate that our test strip system is capable of detecting glucose in concentrations as low as 5 mmol/L, and ketones in concentrations as low as 0.5 mmol/L. For comparison, commercial test strips such as "diagnos" and "GRF" urinalysis test strips are advertised as capable of detecting glucose at concentrations as low as 5 mmol/L and ketones at concentrations as low as 0.1 mmol/L. Moving forward, we will seek to improve the sensitivity of our ketone assay such that it is at least as high as that of "GRF" urinalysis test strips. It should be noted, however, that ketone levels in the urine of healthy adults can reach concentrations as high as 0.5 mmol/L, suggesting that our test strips do not necessarily need to be made more sensitive to accurately screen for diabetes.

VI. CONCLUSION

Colorimetric detection assays for metabolites such as glucose and ketones can be efficiently and inexpensively stamped on filter paper and function with comparable specificity and sensitivity to commercial products. The development of an effective and inexpensive preventative disease screening method for diabetes provides an opportunity to significantly impact the developing world. Future work will determine how the sensitivity of glucose and ketone detection assays can be improved. Of additional interest is the durability of detection reagents for glucose and ketones in solution after being stamped onto test strips. Further research will be performed to determine how long screening test strips can be stored while consistently producing accurate results. Experiments will focus on temperature and humidity in relation to product shelf-life. Regarding supply chain, a variety of distribution methods for urinalysis strips are currently being studied in rural and urban Sierra Leone, utilizing two-parameter OEM strips. Results of the study will guide future decisions regarding distribution pathways. In addition, partnership and funding by local NGOs are being considered for the development of urinalysis strips as an established diabetes screening solution in Sierra Leone. Moving forward, additional research into more "stampable" assays using our system to detect a host of other noncommunicable and communicable diseases prevalent in the developing world, such as UTIs, will also be pursued.

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