



Use of multiple colorimetric indicators for paper-based microfluidic devices

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ARTICLE INFO

Article history:

Received 16 March 2010

Received in revised form 15 June 2010

Accepted 16 June 2010

Available online 25 June 2010

Keywords:

Paper-based microfluidics devices

Colorimetric detection

Microfluidic paper analytical devices

ABSTRACT

We report here the use of multiple indicators for a single analyte for paper-based microfluidic devices (μ PAD) in an effort to improve the ability to visually discriminate between analyte concentrations. In existing μ PADs, a single dye system is used for the measurement of a single analyte. In our approach, devices are designed to simultaneously quantify analytes using multiple indicators for each analyte improving the accuracy of the assay. The use of multiple indicators for a single analyte allows for different indicator colors to be generated at different analyte concentration ranges as well as increasing the ability to better visually discriminate colors. The principle of our devices is based on the oxidation of indicators by hydrogen peroxide produced by oxidase enzymes specific for each analyte. Each indicator reacts at different peroxide concentrations and therefore analyte concentrations, giving an extended range of operation. To demonstrate the utility of our approach, the mixture of 4-aminoantipyrene and 3,5-dichloro-2-hydroxy-benzenesulfonic acid, *o*-dianisidine dihydrochloride, potassium iodide, acid black, and acid yellow were chosen as the indicators for simultaneous semi-quantitative measurement of glucose, lactate, and uric acid on a μ PAD. Our approach was successfully applied to quantify glucose (0.5–20 mM), lactate (1–25 mM), and uric acid (0.1–7 mM) in clinically relevant ranges. The determination of glucose, lactate, and uric acid in control serum and urine samples was also performed to demonstrate the applicability of this device for biological sample analysis. Finally results for the multi-indicator and single indicator system were compared using untrained readers to demonstrate the improvements in accuracy achieved with the new system.

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1. Introduction

Point-of-care testing (POCT) has become relatively commonplace in developed nations as a way to augment traditional medicine and increase patient compliance [1]. POCT is also needed in developing nations because it can reduce the number of clinical visits, decrease costs to the patient and healthcare system, increase patient satisfaction, improve clinical outcomes, and provide clinical services for people in low resource settings [2–5]. Paper strip tests, termed lateral-flow immunochromatographic tests, are currently used in these scenarios [6]. Paper strip tests are commercially available for pregnancy [7], diabetes [8,9], drugs of abuse [10,11], and biomarkers of pathogens test [12,13]. Most paper strip tests use visible color changes for qualitative analyte detection. In the assay, flow is directed along the paper matrix by capillary force, and the analyte is subsequently bound by the capture antibody at the test line. However, qualitative analysis is not sufficient when analyte levels are important for diagnosis or treatment. Much effort has

therefore been directed towards the development of quantitative paper strip tests but these devices still required instrumentation and trained personnel for use and are limited to a single analyte [14,15].

As an alternative to traditional paper-based immunochromatographic tests, Whitesides and coworkers introduced paper-based microfluidics (μ PAD), which represent the next generation of paper strip test devices [16–18]. This approach, which combines many advantages of paper strip tests with the utility of microfluidics, holds significant potential for non-instrumented POCT due to its low cost, multianalyte capability, low sample volume, and inherent portability [16–21]. To date, μ PADs have been developed for glucose, protein, lactate, uric acid, and cholesterol determination [16,21]. The results of the assay were quantified by comparing the color intensities generated by unknowns to those generated for known analyte concentrations. Matching color and intensity by eyes can be complicated by many factors, however, including different color perception, differences in lighting, and the difference between the colors of a dry printed color and those seen in wetted paper. In an effort to conduct quantitative analysis for diagnostic tests based on paper microfluidics, several authors have used cameras or scanners to record the color intensity [18]. Camera

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phones and portable scanners can be used by unskilled personnel in remote areas but require transmission of data from on-site to remote experts, delaying the decision making process. Moreover, the intensities of digital images from a camera are affected by the lighting.

One approach to overcome the limitations of colorimetric approaches for μ PADs is to use multiple indicators for a single analyte. Greater visual discrimination is possible when more than one color is developed as opposed to different hues or intensities of a single color [22]. Hence, multiple indicators should provide more accurate results as compared to single color tests by allowing differences in hue and intensity to be averaged across multiple detection spots for the same analyte. In addition, the improved accuracy should allow for diagnosis by untrained personnel without the need to transmit the results to a central laboratory. Here, we report the development of a novel multiple-indicator approach for μ PADs that allows simultaneous detection using iodide [16], the mixture of 4-aminoantipyrine and 3,5-dichloro-2-hydroxybenzenesulfonic acid [23], *o*-dianisidine [24], acid yellow and acid black for each of the three analytes. The devices share advantages of previous μ PADs, while allowing for more accurate quantitative analysis of glucose, uric acid, and lactate without external instrumentation.

The colorimetric assays in this work utilize oxidase enzymes to decompose analytes and produce hydrogen peroxide [23,25]. Hydrogen peroxide then oxidizes the indicators to generate a visible color change. Each indicator yields a different color and will also change color at different analyte concentrations, allowing a greater dynamic range to be achieved [26–28]. Here, the volume of reagent and sample spotted on the devices was optimized first. Dynamic ranges were then investigated. Our approach was successfully applied to quantify glucose (0.5–20 mM), lactate (1–25 mM), and uric acid (0.1–7 mM) in clinically relevant ranges. Finally, the devices were successfully applied to the analysis of control serum and urine samples. To demonstrate the improvements in accuracy of measurement for clinical samples, 10 random untrained individuals were asked to screen μ PADs comparing single indicator versus multiple indicators. Tests using multiple indicators yielded a statistically significant improvement in accuracy of the measurement compared to tests performed with a single indicator color.

2. Experimental

2.1. Materials and equipments

D-(+)-Glucose (99.5%), sodium L-lactate (98%), uric acid (99%), glucose oxidase (from *Aspergillus niger*, 215 U mg⁻¹), uricase (from *Candida* sp., 2 U mg⁻¹), peroxidase Type I (from *Horseradish*, 113 U mg⁻¹), 4-aminoantipyrine (reagent grade), 3,5-dichloro-2-hydroxy-benzenesulfonic acid (sodium salt, 99%), and *o*-dianisidine dihydrochloride (purified grade for use with peroxidase reaction) were purchased from Sigma–Aldrich (St. Louis, MO). Lactate oxidase (from *Aerococcus viridians*, 38 U mg⁻¹) was obtained from A.G. Scientific, Inc. (San Diego, CA). Potassium phosphate (ACS grade), potassium iodide (ACS grade), sodium hydroxide (ACS grade) and acid yellow 34 (Indicator grade) were purchased from Fisher Scientific (Pittsburgh, PA). Acid Black 1 (Indicator grade) was purchased from Acros Organic (Geel, Belgium). Trehalose dehydrate (HPLC grade) was obtained from Calbiochem (Gibbstown, NJ). Acetone (AR grade) was obtained from Mallinckrodt chemicals (Phillipsburg, NJ). SU-8 3025 negative photoresist was purchased from MicroChem Corp. (Newton, MA). Whatman #1 filter paper was obtained from Cole-Parmer (Vernon Hills, IL). All chemicals were used as received without further purification. A digital camera (coolpix5000, Nikon corp.) was used to photograph results.

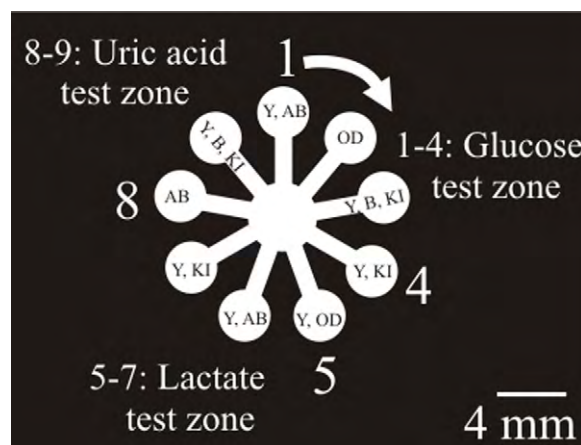


Fig. 1. Design of multiple oxidative indicators for paper-based microfluidic devices which determine three analytes simultaneously with nine test zones. Back and white colors refer to hydrophobic and hydrophilic area, respectively. The device size is 2 cm × 2 cm. Position numbers 1–4: glucose test zones, 5–7: lactate test zones, 8–9: uric acid test zones.

2.2. Preparation of paper-based microfluidic devices

Photolithography was used to pattern the filter paper according to previously reported methods [16,21]. Briefly, SU-8 3025 photoresist was poured on the center of the paper and distributed using a spin-coater (Laurell Technologies Corp., WS-400A-6NPP/LITE). The photoresist-covered paper was baked at 95 °C for ~5 min. The paper was then covered with a patterned transparency film generated using a standard laser printer and irradiated with a UV lamp at 100% intensity (400 W) for 7 s (Uvitron international, Intelli-RAY 400). After baking at 95 °C for ~3 min, unpolymerized photoresist was removed from the paper by submerging in acetone for 1 min, followed by rinsing with acetone. After that, the paper was dried under ambient conditions for approximately 1 h. Prior to adding reagents, the paper microfluidic devices were exposed to an air plasma (Harrick PDC-32G) at 18 W for 30 s. Areas covered with photoresist remained hydrophobic while areas without photoresist were hydrophilic.

2.3. Design of multiple oxidative indicators for paper-based microfluidic devices

For the current experiments, the dendritic flow channels terminating in nine detection zones (giving position number 1–4, 5–7, and 8–9 of the detection zones for glucose, lactate, and uric acid detection, respectively) connected to a central sample deposition spot were created as shown in Fig. 1. Each detection zone was spotted with a different indicator in addition to the appropriate enzyme. The indicators used here were the mixture of 4-aminoantipyrine (AAP) and 3,5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS) in the mole ratio of 1:2 abbreviated to AB, *o*-dianisidine dihydrochloride (OD), potassium iodide (KI), acid yellow 34 (Y), and acid black 1 (B). After oxidation AB, OD, and KI will change from colorless to red [23,29], green-brown [30,31], and yellow-brown colors [16,32,33] while Y and B will change from yellow and black color to colorless, respectively. For detection, sample was added to sample deposition spot and flowed outward via capillary forces to the detection zones.

2.4. Effect of reagent and sample volume

Varying volumes of red food dye (0.4, 0.5, and 0.6 μ L) were dropped into the detection zone to optimize the volume of reagent. The effect of sample volumes was studied by dropping 5.0, 7.0,

9.0, and 11 μL of red food dye into the center of devices using a micropipette. The micropipette which was used to transfer and control the sample volume into device may not be available in the field. In our effort to conduct quantitative analysis for self-monitoring diagnostic tests based on paper microfluidic devices without external equipment, the assays of known volume of level 4 standard solution (11 μL) containing 5 mM glucose, 10 mM lactate, and 4.5 mM uric acid were compared with unknown volume dropped by dropper. Images of fully developed tests were captured with a digital camera for additional characterization.

2.5. Preparation of multiple oxidative indicators for paper-based microfluidic devices

For a given analyte, positions 1–4, 5–7, and 8–9 were spotted with glucose oxidase, lactate oxidase, and uricase enzyme, respectively and the different indicators. The glucose assay was prepared by spotting 0.5 μL of mixture each oxidative indicator, glucose oxidase solution (645 U mL^{-1}), *Horseradish* peroxidase (339 U mL^{-1}), and 0.3 M trehalose into the four detection areas of glucose. Trehalose is added to stabilize the enzyme according to prior reports [34]. The three lactate and two uric acid test zones were created with the different component of indicators and the same amount of *Horseradish* peroxidase and trehalose but the specific enzymes used were lactate oxidase (114 U mL^{-1}), and uricase (80 U mL^{-1}), respectively. The indicator composition of each test zone is shown in Fig. 1. After spotting the reagent solution, the paper was allowed to dry at room temperature ($\sim 22^\circ\text{C}$) for 10 min. All standard and enzyme solutions were prepared in 0.1 M potassium phosphate buffer (pH 6) except for 15 mM of uric acid stock solution which prepared in 20 mM sodium hydroxide. 6 levels of standard solution containing with glucose, lactate, and uric acid and a negative control consisting of buffer were used to study the multiple indicator approach. The concentrations of glucose, lactate, and uric acid in each level of standard solution are shown in Table 1.

2.6. Human sample

Human control serum and urine samples (levels I and II) were obtained from Pointe Scientific (Canton, MI) and Quantimetix Corporation (Redondo Beach, CA), respectively. These complex samples are intended to mimic biological fluids and are used to validate clinical assays commercially. Analyte concentrations were provided by the supplier. All samples were analyzed using multiple oxidative indicators for paper-based microfluidic devices without pretreatment. To test the hypothesis of improved accuracy, results from urine and serum samples were interpreted by 10 randomly selected chemistry students who were unfamiliar with this project for both single and multiple-indicator systems.

2.7. Lifetime of the devices

The devices spotted with oxidase enzyme and indicators solution were kept at 8°C , room temperature ($\sim 22^\circ\text{C}$), and 40°C for multiple days to determine lifetime. Stored devices were tested for lifetime using the standard mixtures (Table 2) every 4 days.

Table 1
Multiple indicators and position of test zones.

Position number	Glucose test				Lactate test			Uric acid test	
	1	2	3	4	5	6	7	8	9
Multiple Indicators	Y, Q	OD	Y, B, KI	Y, KI	Y, OD	Y, Q	Y, KI	Q	Y, B, KI

Table 2
Concentrations of glucose, lactate, and uric acid in each level of standard solution.

Std. level	Glucose Conc. (mM)	Lactate Conc. (mM)	Uric acid Conc. (mM)
0	0	0	0
1	0.5	1	0.1
2	1.5	2.5	1
3	3	5	2.5
4	5	10	4.5
5	10	20	6
6	20	25	7

3. Results and discussion

3.1. Colorimetric bioassays

The goal of this work was to test the hypothesis that multiple indicators for a single analyte would improve the accuracy of the data interpretation in colorimetric μPAD assays. Three analytes of clinical relevance (glucose, lactate, and uric acid) were tested using multiple indicators (AB, OD, KI, Y, and B) for each analyte. The device design is shown in Fig. 1. A combination of indicators was chosen for this assay because of the potential to provide greater visual discrimination than one color. Two general types of indicators were used. The first type generated a stable color on oxidation. AAP and DHBS produced a red quinone product on reaction with peroxide. In a similar fashion, green-brown and yellow-brown stable colors were generated from the reaction of H_2O_2 with OD and KI. The second type of indicator was selected to lose color when oxidized. Here, we used Y (yellow color) and B (blue color) as indicators because they are colorless when oxidized. The relevant indicator reactions are shown in Fig. S1. The indicators are mixed in each test zone so the change in shade and color intensity occurs at the different levels of analyte. For example, we used the mixture of AB as the first indicator and Y as the second indicator at position number 1 for the glucose test. At two different concentrations of glucose, we get two shades of orange and red colors whereas with a single indicator obtains only one shade with different intensity is generated making it more difficult for visual discrimination of the different analyte concentrations. Our proposed method was therefore expected to provide a larger difference in color hues and intensities, allowing better visual discrimination and therefore accuracy. We first studied multiple mixtures as a function of glucose concentration. It was found that glucose required four wells to accurately span the clinical range, where as lactate required three, and uric acid required two. All further studies were performed as shown in Fig. 1 based on this ability to vary range.

3.2. Effect of reagent and sample volume

We first determined the volume of reagent and sample required for analysis. In existing paper test strips, the major cost comes from reagents. Hence, we designed the reaction zones to be small (3 mm diameter) for reduced reagent consumption while still making it large enough to be visible to the naked eye. Reaction zone diameters smaller than 3 mm were studied. Even though the reagent consumption can be reduced with a small detection zone, dispensing less than 0.2 μL is difficult with standard micropipettes. We next determined the reagent volume necessary to wet the entire detec-

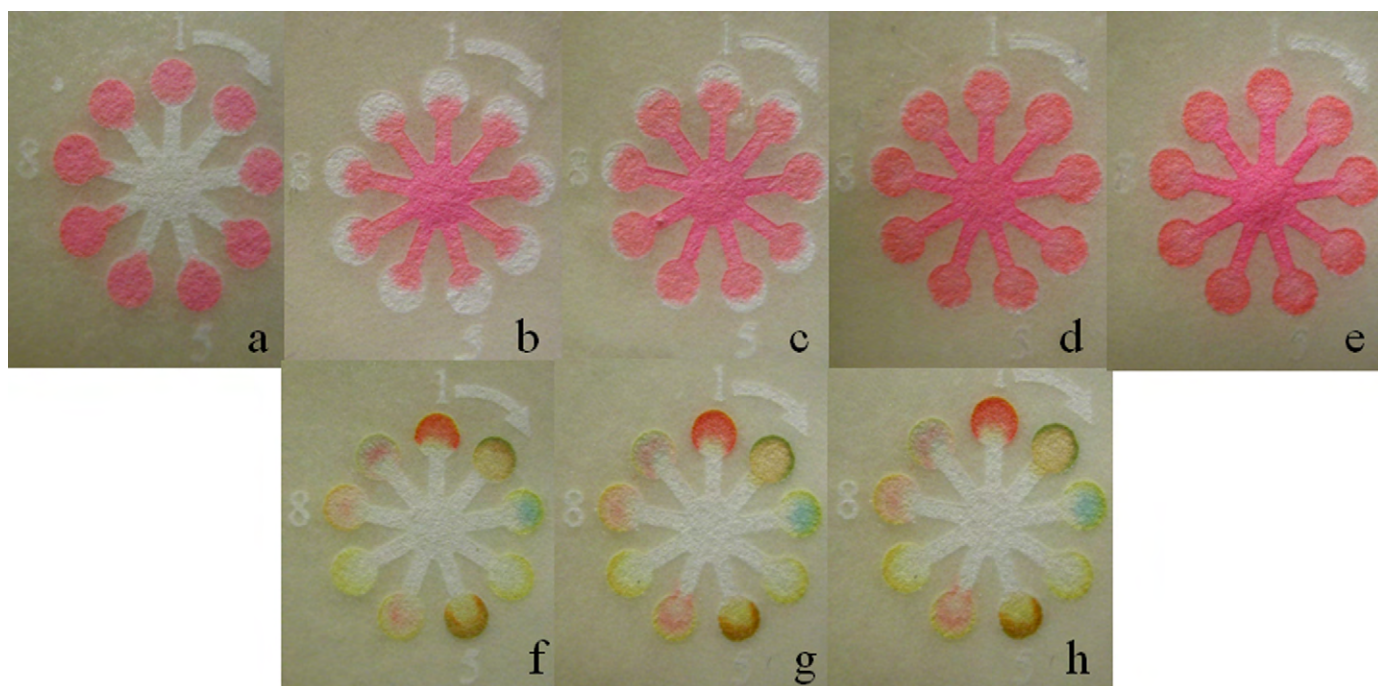


Fig. 2. (a) Paper-based microfluidic devices after spotting red food dyes of various volumes (0.4, 0.5, and 0.6 μL) into the position numbers 1–3, 4–6, and 7–9, respectively of the detection zones. (b–e) Paper-based microfluidic devices after spotting 5 (b), 7 (c), 9 (d), and 11 μL (e) of red food dye respectively into the central of devices. (f–h) Multiple oxidative indicator for paper-based microfluidic devices after spotting level 4 of standard solution including 5 mM glucose, 10 mM lactate, and 4.5 mM uric acid with 11 μL of controlled standard volume (f) and unknown of standard volume (g and h).

tion zone by dropping red food dye in the range of 0.4–0.6 μL into the detection zones. As the results show in Fig. 2a, 0.4 μL of reagent solution cannot completely wet the detection zones whereas 0.6 μL spread outside the detection zones. Therefore, we selected 0.5 μL of reagent solution for the further experiments. The minimum sample volume that can spread through the entire device was also studied by spotting red food dye into the center of the device. It was found that 11.0 μL of sample is required to fill all detection zones (Fig. 2b–e). In many situations a micropipette may not be available to apply an accurate sample volume. Hence, the effect of sample volume on the assays reaction was determined by dropping a standard solution level 4 containing 5 mM glucose, 10 mM lactate, and 4.5 mM uric acid into the center of the device with 11.0 μL . In addition, an unknown volume was dispensed with a disposable transfer pipet to simulate real field-testing. The results with controlled volumes (Fig. 2f) were compared with results from unknown volumes (Fig. 2g and h). We found that the color hue of the three indicators was the same regardless of the sample volume added, meaning

tight control of sample volume was not necessary. In the field, the samples can therefore be directly dropped into our devices using simple transfer pipettes and similar spotting devices.

3.3. Simultaneous measurement of three analytes

The principle of our proposed method is to use the color change of each oxidative indicator and the color intensity at different analyte concentrations to improve the accuracy and extend the linear range of colorimetric μPAD assays. To demonstrate the multiple-indicator systems, we studied the dynamic range of glucose, lactate, and uric acid on a single device. The results were captured with a digital camera for visualization (Fig. 3). Glucose at 0.5 mM generated an orange color (position number 1). Glucose at 1.5 mM showed orange and green-brown colors, while glucose between 3 and 5 mM gave red and green-brown colors (position number 1 and 2, respectively). Glucose concentrations ≥ 10 mM glucose gave red, green-brown, and brown colors (position numbers 1–4). Moreover,

Std. level	0	1	2	3	4	5	6
Conc. (mM)							
Glucose	0	0.5	1.5	3	5	10	20
Lactate	0	1	3	5	10	20	25
Uric acid	0	0.1	1	2.5	4.5	6	7

Fig. 3. Multiple oxidative indicators system designed on paper-based microfluidic devices for the simultaneous semi-quantitative analysis of glucose, lactate, and uric acid. Pictures were captured after spotting varying concentration of three analytes for 10 min.





Samples		Certified Concentration (mM)	Our proposed method (mM)	
Serum Level I	Glucose	5.6		5-10
	Lactate	1.2		1
	Uric acid	0.2		0.1
Serum Level II	Glucose	16.8		20
	Lactate	3.3		2.5
	Uric acid	0.7		1
Urine level I	Glucose	2.9		3-5
	Lactate	Not labeled		ND
	Uric acid	0.5		0.1
Urine level II	Glucose	16.5		20
	Lactate	Not labeled		ND
	Uric acid	1.1		1

Fig. 4. Multiple oxidative indicators system designed on paper-based microfluidic devices for the simultaneous semi-quantitative analysis of glucose, lactate, and uric acid in real biological samples.

the intensity of red, green-brown increased when glucose concentrations increased. Hence, the difference of hue and intensity of multiple indicators can be used to identify glucose concentration. The normal level of glucose is 2.5–5.3 mM in serum, and 0.1–0.8 mM in urine [35]. Given the dynamic range of the color changes, our devices could be used in a variety of biological matrices such as serum, plasma, and urine.

Our device also gave multiple color hues for different concentrations of lactate. Lactate at 1 mM gave brown and orange colors, while lactate concentrations between 5 and 20 mM gave green-brown and red colors at positions 5 and 6, respectively. Moreover, lactate concentrations ≥ 25 mM showed three colors, green-brown, red, and brown at positions 5, 6, and 7, respectively. These devices are therefore sufficient for clinical diagnostics where the normal concentration of lactate is 0.5–1.7 mM in serum, and 5.5–22 mM in urine [35].

Uric acid detection zones also exhibited color changes as a function of concentration. Concentrations at 0.1 mM gave orange color at position number 8, while concentrations between 1 and 2.5 showed orange and red colors at position number 8 and 9, respectively. At uric acid concentrations ≥ 2.5 mM, both position number 8 and 9 were red. The normal level of uric acid is 0.1–0.4 mM in serum and 1.5–4.4 mM in urine [35]. These results clearly suggest the ability to visually discriminate between different concentrations using multiple indicators for a single analyte. The reproducibility of our proposed method was also studied by spotting each level of standard solution into three paper devices in the same day (intra-day

assay) and three different days (inter-day assay) (Fig. S2). At each test zone, the change in color intensity as a function of analyte concentration was obtained with high reproducibility.

3.4. Semi-quantitative measurement of three analytes in real samples

The multi-indicator μ PAD devices were next evaluated for glucose, lactate, and uric acid analysis in clinical control samples. The control samples are used for determining the accuracy of diagnostic tests in a biologically relevant matrix without worry of blood borne pathogens. The results are shown in Fig. 4. We found that level I serum and urine samples gave significantly different color patterns than the level II samples. The results indicated that glucose concentrations were between 5–10 mM in serum level I (control level: 5.6 mM), and 3–5 mM (control level: 3.3 mM) in urine level I, whereas 20 mM glucose was determined in both serum and urine samples level II (control serum and urine level: 16.8 and 16.5 mM, respectively). For the lactate test, we obtained brown and orange colors for both serum sample levels I and II. This indicated lactate levels in both serum sample levels were between 1–2.5 mM. Moreover, the intensities of brown color from standard lactate can be used to identify lactate concentration of 1 mM in level I (control level: 1.2 mM) and 2.5 mM (control level: 3.3 mM). In these cases, both the color intensity and hue can be used to confirm our results. Lactate test zones of both urine sample levels show insignificant difference of the color changing comparing with buffer solution

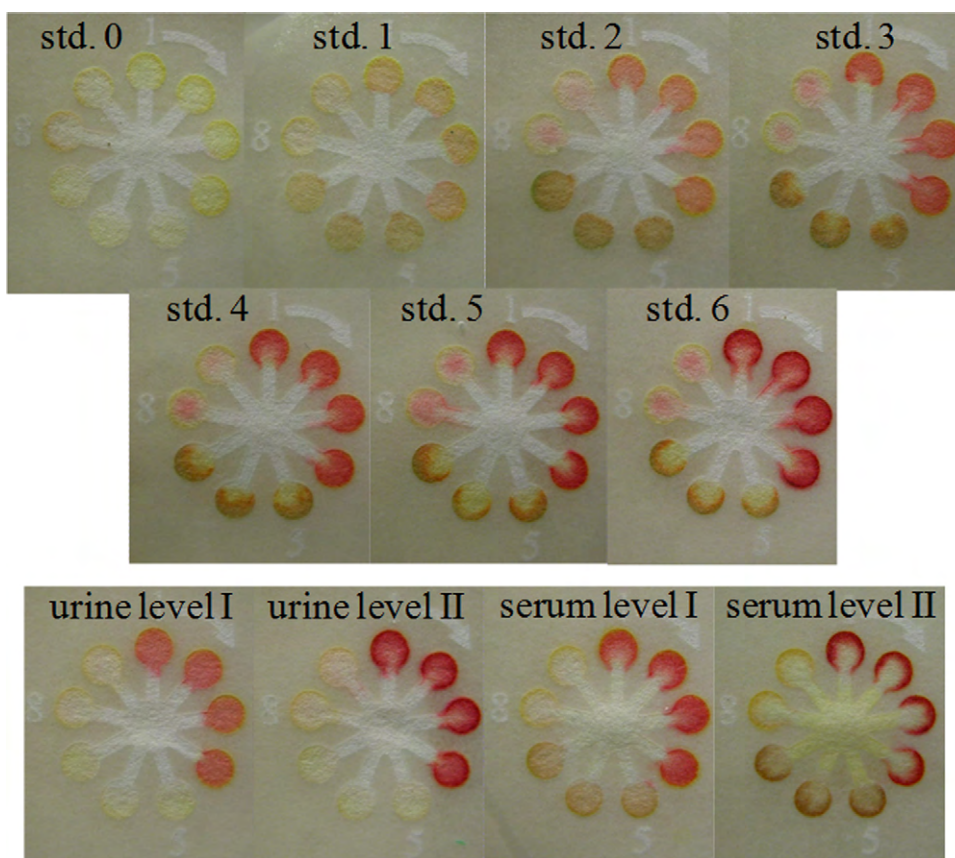


Fig. 5. Single indicator system designed on paper-based microfluidic devices for the simultaneous semi-quantitative analysis of glucose, lactate, and uric acid. Position numbers 1–4: glucose test zones using Y + AB indicator, 5–7: lactate test zones using Y + OD indicator, 8–9: uric acid test zones using AB indicator.

(standard level 0). Therefore, we can indicate both urine samples in the absence of lactate correlated with the certificated concentration. Uric acid concentrations in samples were determined to be 0.1 mM for level I serum and urine samples (control level: 0.2 mM in serum and 0.5 mM in urine) and 1 mM for level II serum and urine samples (control level: 0.7 mM in serum and 1.1 mM in urine) by comparing the differences in color intensity.

To demonstrate our method comparing with a single indicator system in terms of accuracy, the highest sensitivity single dye indicators including Y + AB, Y + OD, and AB for the glucose, lactate, and uric acid test, respectively were selected for single indicator systems as shown in Fig. 5. We also compared the percentage of correct answer obtained from 10 untrained individuals using single and multiple-indicator tests (Fig. 6). The results indicated that our devices were successfully applied for glucose, lactate, and uric acid screening tests by the naked eye. As can be seen in Fig. 5, there is a clear difference between the two sets of data in terms of colors generated. Furthermore, Fig. 6 shows the increase in accuracy for the tests using multiple indicators. The single indicator system had an accuracy of ~70%, while the multi-indicator system had an accuracy of over 90%. The results suggest our approach provides a more accurate result when compared to a single indicator system.

3.5. Lifetime of the devices

Diagnostic devices must remain stable for weeks to be useful in the field for use in developing countries. Hence, the performance of devices was studied after storing the prepared paper devices for multiple days at varying temperatures. To test stability, prepared paper devices were dried at ambient condition before storage at either 8 °C, room temperature (~22 °C), or 40 °C. Oxidase enzymes

can degrade, aggregate, or unfold during dry storage [35]. Non-reducing sugars such as sucrose and trehalose, and polyols such as mannitol have been used to stabilize dried proteins during storage [34,36–38]. Here, trehalose was added to oxidase enzymes solution during devices preparation to improve stability of the enzyme during storage. The lifetime of these devices was observed over a period of several days as shown in Fig. S3. We found that multiple indicators, which were generated at all detection zones of all standard solution levels, exhibited no significant difference from day to day at all temperatures. At 12 storage days at room temperature and 40 °C, an observable signal decrease was noted. The

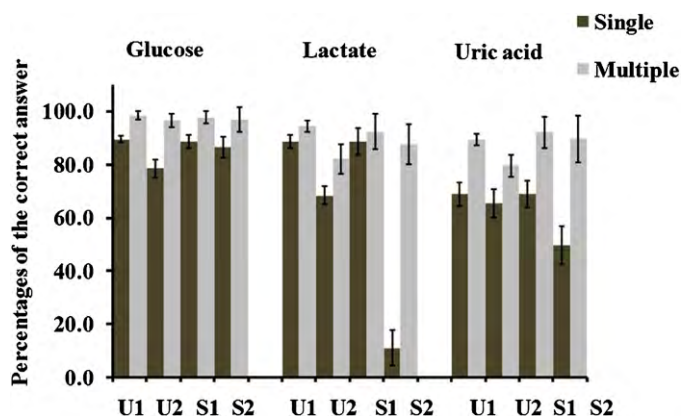


Fig. 6. Comparison of percentages of the correct answer where a corrected answer was determined to be within ± 0.5 mM of the certified concentration between single and multiple-indicator systems ($n = 10$). U1: level I urine human, U2: level II urine human, S1: level I serum human, S2: level II serum human, error bar: standard deviation ($n = 3$).

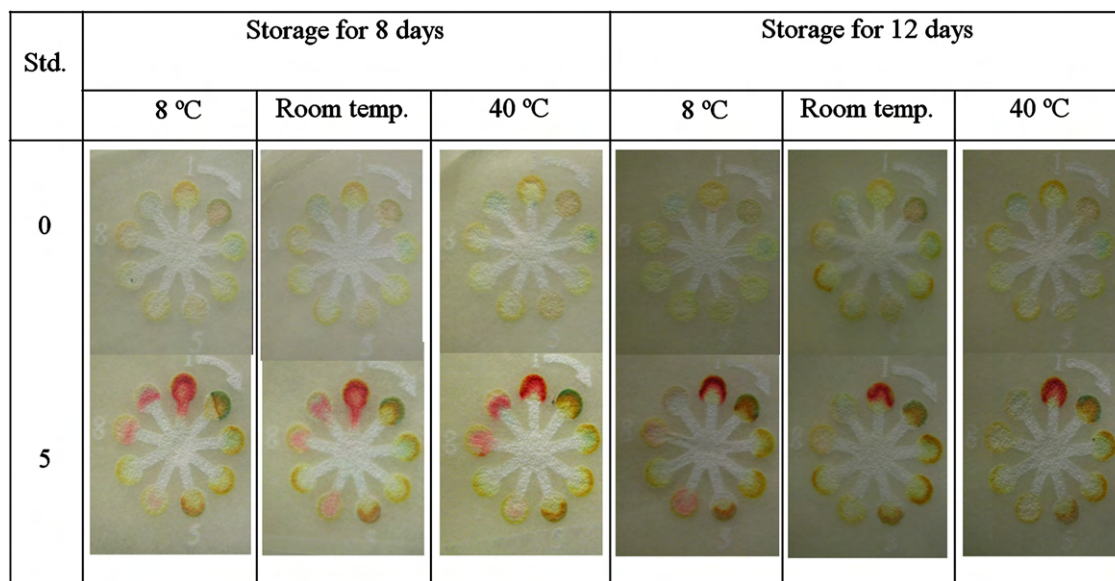


Fig. 7. Lifetime of our devices kept at 8 °C, room temperature (~22 °C), and 40 °C. Background signals were obtained by spotting 0.1 M of phosphate buffer solution while standard test signals were obtained by spotting 10 mM glucose, 20 mM lactate, and 6 mM uric acid.

results indicated the devices can be kept for 8 days without loss of activity but longer storage time requires refrigeration (Fig. 7). Future work will focus on methods to increase the lifetime of these devices to allow months of storage at elevated temperatures.

4. Conclusion

This paper demonstrates the use of multiple indicators for a single analyte as part of a multianalyte μ PAD. Multiple indicators improve accuracy of detection by improving the ability to visually discriminate between different concentrations. While different concentrations of the same dye could also be used to achieve a similar result, using different colors provides increase visual discrimination capability. Furthermore, different indicators generate colors at different analyte concentrations, which should provide more accuracy than different shades or intensities of a single color. Our devices were also successfully applied to the simultaneous semi-quantitative analysis of glucose, lactate, and uric acid in biologically relevant samples. These results demonstrate the feasibility of using multiple oxidative indicators for paper-based microfluidic devices as an easy-to-use, inexpensive, and portable alternative device for point-of-care testing and self-monitoring diagnosis.

Acknowledgements

WD gratefully acknowledges the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (Grant No. PHD/0039/2548). OC also would like to thank the Thailand Research Fund (Basic Research Grant) and the Thai Government Stimulus Package 2 (TK2555), under the Project for Establishment of Comprehensive Center for Innovative Food, Health Products and Agriculture (PERFECTA). CSH thanks support through the Fulbright Scholar program through the US-Thailand Education Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2010.06.019.

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