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Development of Field Deployable Point-of-Care Diagnostic Systems

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Abstract. Existing technology in the form of Vitros™ dry slides, marketed by Johnson & Johnson via the Ortho Clinical Diagnostics division, was employed to explore various factors crucial to the development of a field deployable reflectance colorimeter. These factors include: sample volume, reaction kinetics, incubation temperature, and method of data collection and analysis. Vitros™ glucose, and urea (BUN) dry chemistry slides were used during the investigation. Various detection methods including an iPhone® using a third party app, ImageJ, as well as Variable Technologies' Node Chroma™ were used to evaluate color development. Our investigation of sample volume illustrates that despite the requirement of 10 μL specified by Ortho, smaller volumes (>4 μL) can produce results that are equally as precise. Our efforts in temperature and kinetics highlight the need for an integrated heating component in the final device. Finally, lighting was also studied and demonstrated that a single light source is not acceptable; rather multiple light emitting diodes (LEDs) are necessary to provide the even lighting that is crucial for accurate color measurement.

Keywords: Point-of-care, Chronic diseases, Early diagnosis, Dry chemistry assay, Portable sensor

1. Introduction

Chronic diseases are increasing in global prevalence and seriously threaten the ability of developing nations to improve the health of their populations. The global health landscape is rapidly shifting away from one dominated by infectious diseases to one characterized by various chronic conditions, including cardiovascular disease, kidney failure, cancer, and diabetes. These chronic conditions now cause more than half of all deaths worldwide, 80% of which occur in low-income, developing countries. Despite accounting for more than 60% of all deaths worldwide, chronic diseases are surprisingly neglected on the public health agendas of many developing nations. [1] With the majority of chronic diseases being known, many of these deaths are preventable. These diseases are treatable, but often go undiagnosed. Early diagnosis and disease management is key to slowing disease progression and maintaining an ability to function in everyday life, which is especially critical for low-income families. The objective of early diagnosis is the early detection of asymptomatic diseases when intervention has a reasonable potential to have a positive impact. However, with nearly a third of all countries (mostly African and Asian) spending less than USD $100 per capita on healthcare annually, the ability to diagnose these chronic diseases cannot be taken for granted.[2]

Many chronic diseases are first identified and later diagnosed through standard clinical chemistry tests. However, the resources and the skilled personnel to conduct these tests are not available in many developing nations. Many researchers are focusing on developing paper-based assays destined for developing nations. [3]-[7] There are already a large number of clinical chemistries on the market in a dry reagent slide format covering various regimes including hepatic, cardiac, renal, diabetic and general chemistries. The Ektachem™ series of diagnostic test products, originally produced by Eastman Kodak and now marketed by Johnson & Johnson under their Ortho division as Vitros™ dry slides, are based on many of the same concepts being used by paper microfluidic researchers today. Kodak produced a complete clinical chemistry analyzer menu based on the reflectance spectrophotometric detection of dyes produced during the course of enzymatically driven reactions. These tests were conducted on 16 mm^2 dry reagent slides that in many ways are similar to

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formats being advocated today for 3rd world applications. Although the Ortho marketed implementation of this technology is too expensive as currently designed (both instrument capital cost (>$100,000, and cost per test of the consumable components ($1-5 per test)), it points the way for those interested in adapting the basic concepts to the challenges of developing nations. The first step to making these commercially available clinical chemistry tests widely available is the development of a new reflectance colorimeter. The new colorimeter will utilize off the shelf components and preliminary estimates put the cost at less than $50. In this study a few of the many variables associated with developing a new reflectance colorimeter to analyze Ortho's Vitros™ slides are investigated.

2. Experimental

2.1. Materials

D-(+)-Glucose (99.5%), Sodium phosphate monobasic (anhydrous), sodium phosphate dibasic (anhydrous), sodium chloride, sodium hydroxide, and urea were purchased from Sigma-Aldrich (St. Louis, MO). Ortho Clinical Diagnostics Vitros™ slides for glucose and urea (BUN) were purchased from Fisher Scientific (Waltham, MA). Deionized water was obtained from a Milli-Q Advantage A10 ultrapure water purification system from EMD Millipore (Billerica, MA). An iPhone® 4s complete with an 8-megapixel camera was used throughout this study. For controlled lighting, an ipics2go photo scanner box (ION) was used. The NODE Chroma™ was purchased from Variable Technologies (Knoxville, TN) and used as received.

2.2. Sample Preparation

For tests using Vitros™ Glucose dry slides, glucose solutions were prepared by dissolving D-glucose in a phosphate buffer (0.01M NaH2PO4, 0.01M Na2HPO4, and 0.15M NaCl brought to pH 7.0 with 1.0M NaOH). Concentrations ranging from 40 mg/dL to 400 mg/dL were prepared by creating a stock solution of 400 mg/dL and subsequently diluting to achieve the desired concentration.

For tests using Vitros™ BUN dry slides, urea solutions of clinically relevant concentrations were prepared by dissolving urea (Sigma-Aldrich U0631) in deionized water. Concentrations ranging from 15 mg/dL to 45 mg/dL were prepared by creating a stock solution of 45 mg/dL and subsequently diluting to achieve the desired concentration.

2.3. Sample Volume Study

In order to understand the effects of sample volume on the colorimetric development of the Vitros™ dry slides, various sample volumes (0.25 - 30 μL) of 400 mg/dL glucose solutions was applied to the center of the slide. The slides were then allowed to incubate for 5 minutes at room temperature before an image was captured with an iPhone® under controlled lighting. Jpeg images were then analyzed using the 3D interactive surface plot plugin as well as the RGB profiler plugin in ImageJ. Subsequent images were captured after 1 hour of incubation at room temperature and subjected to the same analysis.

2.4. Temperature Study

Temperature plays a role in enzyme kinetics. In order to understand the importance of temperature in dry slides, Vitros™ BUN slides were analyzed under varying temperatures to see the effects on color development. 5.5 μL of 15 mg/dL urea was applied to the slide and incubated at 22°C, 37°C, 45 ℃, and 60 ℃ for 30 minutes. During the incubation process, color intensities were recorded every 60 seconds using the Node Chroma™.

2.5. Data Analysis

The color intensities of various Vitros™ slides were quantified with one of three methods. First, images were captured using an iPhone® and subsequently analyzed using the software platform ImageJ. Second, a third party app, ColorAssist, was used to analyze the developed color directly on the iPhone®. Finally, the Node Chroma™ was used in combination with the iPhone®. All of these methods make use of a 256-bit color scale in which a value of zero represents an absence of color and a value of 256 represents the maximum presence of color. Each method reports color in three channels; red, green, and blue.

3. Results and Discussion

3.1. Lighting

Vitros™ slides operate based on reflectance colorimetry meaning that accurate measurements can only be made when consistent lighting and environmental conditions are met. A wide range of lighting layouts are integrated in the detection methods we have used. The NODE Chroma™ is equipped with a ring of 6 LEDs, while the iPhone® is equipped with a single broadband source (flash). We have found that a single light source directed at various angles (30, 45, 60, and 90 °) was not sufficient to produce an even color contrast on the slide (Fig. 1). Because of uneven color contrast the location of the measurement and the sample positioning become extremely important. Integration of multiple external LEDs perpendicular to the slide surface, or in a circular pattern encompassing the detection zone provides even lighting of the slide surface and allows for more forgiving sample positioning and increased accuracy.

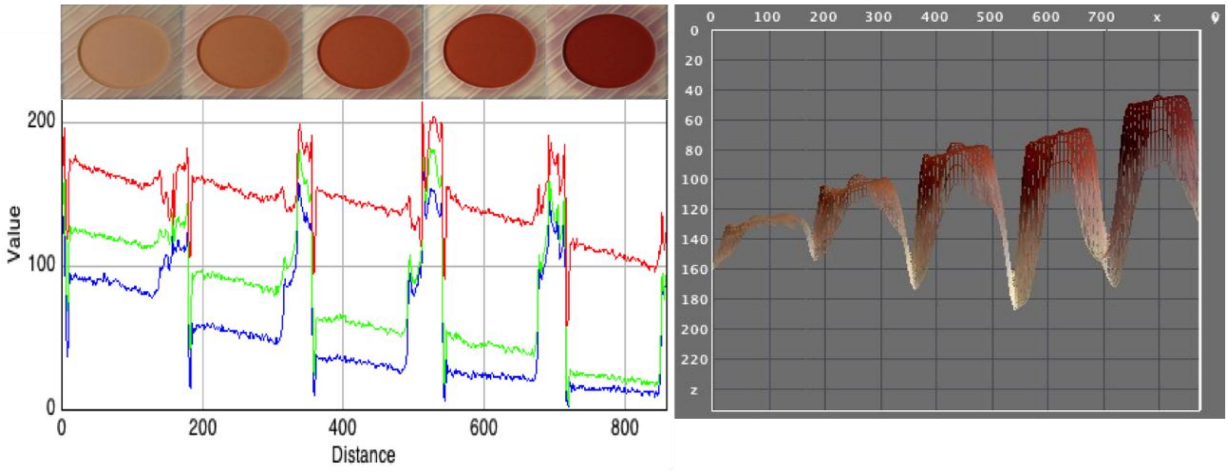

Fig. 1: 3D surface plot and RGB profile of 10 µL samples of 40, 100, 200, 300, and 400 mg/dL glucose analyzed with uneven lighting. A single light source from the left side of the slides was used creating the uneven lighting as apparent in the angled distribution of color. Despite *even color development*, images capture the *uneven lighting*. This leads to uncertainty in measurements based on location and sample positioning.

3.2. Sample Volume

According to Ortho, the Vitros™ glucose dry slides require a sample volume of exactly 10µL in order to yield accurate and precise measurements. However, in the field micropipettes and the skilled personnel to use them are not always available. In addition, 10µL is a relatively large sample volume for a point-of-care device and can be difficult to obtain from a finger prick. As a comparison, a typical glucose meter requires 0.5 -2.0µL. In this study, we are investigating the Vitros™ slide sample volume tolerance.

Samples ranging from 0.25µL to 30µL (the average volume of a drop from a disposable plastic pipette) of 400mg/dL glucose were applied to the center of a slide and allowed to incubate at room temperature for 5 minutes (Fig. 2 A), the same slides were then reanalyzed after 1 hour (Fig. 2 B) Images of each slide were taken with an iPhone® and then analyzed using the 3D surface plot plugin and RGB profile plugin in ImageJ. The surface plots show the variation in the grey scale color intensity (Z axis) over the x-axis and y-axis. Even color distribution was seen with samples as small as 4 µL as seen by the transition of a bell-shaped color distribution at lower volumes to flat, even distribution for volumes of 4µL or greater. This showed that there is some flexibility in the sample positioning in relation to the reflectometer and the specific location of the measurement on the slide.

The RGB profiles of the slides showed that the distribution of the blue channel, previously found to have the most sensitive and linear response to glucose concentration, exhibited slight variations with increasing sample volume. After 5 minutes of incubation, the blue channel had an average reading of 49.15 ± 2.76 (3 to 20 µL). However, after 1 hour the average value was 24.00 ± 2.24 indicating that the slides continued to develop after the suggested five-minute incubation period and that the color became more consistent. Following the addition of large volume samples, the blue channel values increased significantly to 53.57 ±

16.74 after 5 minutes, while only increasing to 24.21 ± 2.29 after 1 hour. These results suggest that pipetting exactly 10 µL may not be absolutely critical depending on the particular circumstances.

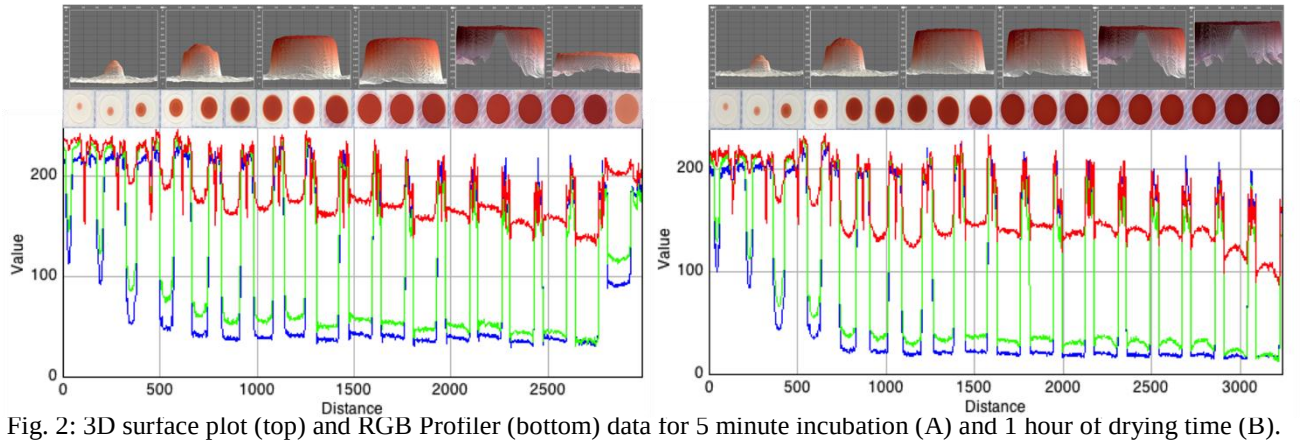

Fig. 2: 3D surface plot (top) and RGB Profiler (bottom) data for 5 minute incubation (A) and 1 hour of drying time (B). 3D surface plot analysis (from left 0.25, 1, 4, 6, 10, 30 µL sample volume) demonstrates that samples as low as 4 µL provide even color distribution. RGB Profiler data and accompanying slide images (middle) for sample volumes (from left) 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 20, 30 µL show relative stability in the blue channel for samples of 3 µL or greater. This stability is increased, especially at higher sample volumes, after 1 hour of incubation.

3.3. Temperature

Ortho's family of analyzers each contain an incubation chamber which heats the slides to 37 ℃ for the duration of the incubation period, typically 5-10 minutes. In a portable device designed for use in remote locations, heating can be problematic due to variations in environmental temperatures, so the effects of incubation were studied to determine its necessity. Tests utilizing Vitros™ BUN/Urea slides were conducted at four temperatures, 22 ℃ (room temperature), 37 ℃, 45 ℃ and 60 ℃. These values were chosen to gain an understanding of the effect of temperature on enzyme kinetics within the dry slide. Fig. 3 shows plots of color intensity (RGB) vs. time, in minutes, for all three color channels at 37 ℃, and the comparison of the green channel, which has the largest dynamic range, for all temperatures. At room temperature, maximum color intensity was observed at approximately 25 minutes. This profile shifted when the temperature was raised. Maximum color intensity was achieved more rapidly at approximately 20 minutes, 15 minutes, and 10 minutes for 37 ℃, 45 ℃, and 60 ℃ respectively. Also, an increase in maximum color intensity was seen with increasing temperature. Both of these results can be interpreted as increased enzyme kinetics due to increased temperature. While increasing the temperature does improve kinetics, it has a drawback in the rapid onset of sample deterioration, seen here as an increase in green intensity. These results demonstrate the necessity for controlled heating in the final colorimeter.

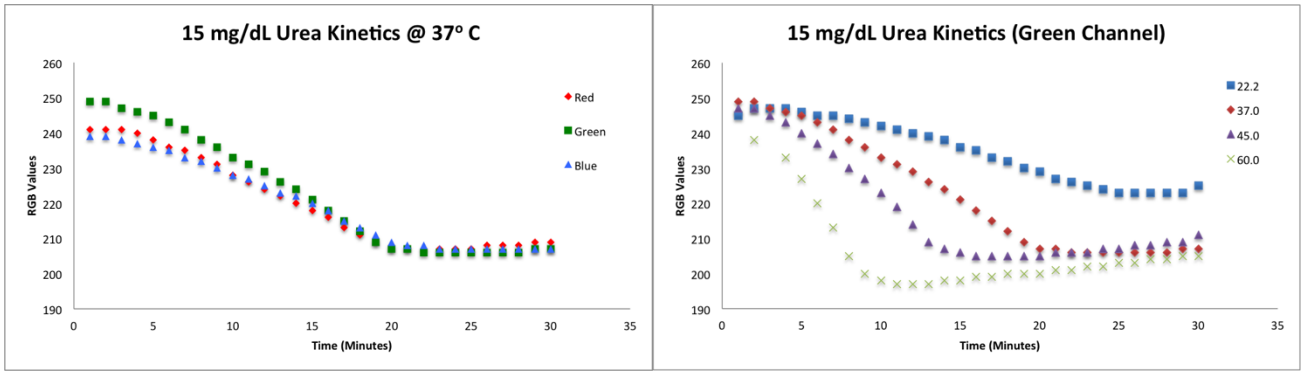

Fig. 3: Kinetics data for all three channels (RGB) of a 15 mg/dL urea sample at 37 ℃ (A), and a comparison of the green channel for 15 mg/dL urea samples at 22 ℃, 37 ℃, 45 ℃, and 60 ℃ (B). At 37 ℃ the green channel shows the largest dynamic range and maximum color intensity is achieved after approximately 20 minutes. This value shifted higher (approximately 25 minutes) at room temperature (22 ℃), and lower (15 and 10 minutes) for 45 ℃ and 60 ℃ respectively. Also, increased color intensity was seen with increasing temperature.

4. Conclusion

Chronic diseases are rapidly overtaking infectious diseases as a leading cause of death in rapidly developing nations. Early diagnosis is key to successful management of chronic diseases, however, many nations lack the infrastructure to diagnose and treat these diseases. Paper microfluidic devices offer a cost effective alternative to modern diagnostic practices, however current manufacturing and analysis of these devices need improvement. By studying Ortho's commercially available FDA approved Vitros™ dry slides we have taken the next step in the development of a portable reflectance colorimeter made from off the shelf components costing less than $50 (USD). We have demonstrated that there are several factors that must be controlled in order to obtain accurate and precise measurements, such as the assay temperature and environmental lighting. While increasing the temperature does lead to faster kinetics and more intense color development, it also speeds of the degradation of the dry slides which makes the timing of the measurements more crucial. Even lighting is crucial for an accurate measurement, incorporating multiple LEDs can help alleviate the issues caused by a single light source. Finally, we have shown that sample volume is not as critical as once thought. We have shown that even with a disposable pipette precise measurements can be taken when only one color channel is used for the analysis and the appropriate amount of drying time is used. The information gained from this study will be used in the fabrication of a portable reflectance colorimeter capable of quantifying clinically relevant analytes with Vitros™ slides as well as other paper microfluidic formats.

5. Acknowledgements

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