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Clinical chemistry measurements with commercially available test slides on a smartphone platform: Colorimetric determination of glucose and urea

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ABSTRACT

Background: Rapidly increasing healthcare costs in economically advantaged countries are currently unsustainable, while in many developing nations, even 50-year-old technologies are too expensive to implement. New and unconventional technologies are being explored as solutions to this problem. In this study, we examined the use of a smartphone as the detection platform for 2 well-developed, relatively inexpensive, commercially available clinical chemistry assays as a model for rapid and inexpensive clinical diagnostic testing.
Methods: An Apple iPhone 4 camera phone equipped with a color analysis application (ColorAssist) was combined with Vitros® glucose and urea colorimetric assays. Color images of assay slides at various concentrations of glucose or urea were collected with the iPhone 4 and quantitated in three different spectral ranges (red/green/blue or RGB) using the ColorAssist app. When the diffuse reflectance data was converted into absorbance, it was possible to quantitate glucose or blood urea nitrogen (BUN) over their clinically important concentration ranges (30–515 mg/dl for glucose or 2–190 mg/dl for BUN), with good linearity ($R^2 = 0.9994$ or 0.9996, respectively [$n = 5$]).
Results: Data collected using the iPhone 4 and canine serum samples were in agreement with results from the instrumental "gold standard" (Beckman Coulter AU480 Chemistry System) ($R^2 = 0.9966$ and slope = 1.0001 for glucose; $R^2 = 0.9958$ and slope = 0.9454 for BUN). Glucose determinations of serum samples made using this smartphone method were as accurate as or more accurate than a commercial colorimetric dry slide analyzer (Heska® Element DC Chemistry Analyzer, Loveland, CO) and 2 glucometers: ReliOn® Ultima (Abbott Diabetes Care Inc) and Presto® (AgaMatrix Inc.H). BUN determinations made using the smartphone approach were comparable in accuracy to the Heska instrument.
Conclusion: This demonstration shows that smartphones have the potential to be used as simple, effective colorimetric detectors for quantitative diagnostic tests, and may be applicable for both point-of-care applications in the developed world and field deployment in developing nations.

1. Introduction

The annual cost of healthcare in the U.S. is anticipated to reach $4.8 trillion or 17.6% of the U.S. GDP in 2021, up from over $2.59 trillion dollars as of 2010 [1]. This rapid increase in healthcare spending is widely regarded as unsustainable. As of 2008, diagnostic testing constituted $250 billion of this budget [2], with a majority share devoted to medical imaging products. A concurrent issue is that traditional healthcare technologies are too expensive to implement in many developing parts of the world, where several dozen nations spend less than $100 per capita annually on healthcare [3].

The nature of many diseases makes frequent monitoring necessary, such as glucose assays for diabetes and blood urea nitrogen (BUN) assays to track kidney diseases. Global healthcare expenditures for diabetes alone are expected to reach $490 billion by 2030, while the number of diabetes cases worldwide will double between 2000 and 2030 to over 400 million people [4,5]. The point-of-care testing (POCT) concept was introduced with these figures in mind. Benefits of POCT include immediate output via an electronic medical record (EMR), decreased turnaround time via enhanced communication efficiency, and reduction of morbidity and mortality. POCT devices are predicted to play a vital role in decreasing healthcare costs.

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Various formats of membrane-based test strips, dry chemistry slides, and paper microfluidic devices, all designed to utilize smaller amounts of samples and reagents while producing faster results (often with enhanced sensitivity) have been examined for medical diagnostics and other applications [6–8]. To further these efforts, parallel projects have been pursued to develop detection approaches that can match the simplicity and low cost of these paper strip approaches. While a variety of detection methods, including electrochemical [9–11], chemiluminescence [12,13], and fluorescence [14,15] have been investigated, comparatively simple colorimetric methods (which have a long history) are still attractive because the output of these assays can be visually observed and compared to a color chart to generate low-cost, rapid, semi-quantitative results. Truly quantitative detection of these paper-based colorimetric reactions requires the use of benchtop scanners [16] or cameras combined with computer-based image analysis software [17,18], or other sophisticated commercially available greyscale or color intensity measuring instruments. Smartphone cameras have tremendous potential to supplant this quantitative approach given their onboard processing power, portability, widespread availability, internet connectivity, and open source programming [19–23]. The built-in digital camera of the iPhone, for example, combined with widely available software (Adobe Photoshop) has been applied to field tests for colorimetric trinitrotoluene (TNT) detection [24]. The performance of the built-in camera within the iPhone was compared to that of a digital single lens reflex (DSLR) camera, which resulted in a successful demonstration of the phone camera for semi-quantitative field-testing for TNT explosives.

2. Materials and methods

2.1. Chemistry of dry chemistry products

Dry chemistry slides for clinical glucose (Fig. S1a) and urea (Fig. S1c) assessments were purchased from Vitros® (Ortho Clinical Diagnostics, Johnson & Johnson, Inc.). These slides were typically made of several chemical layers, each of which was designed to optimize specific unit operations as described below.

Glucose slides are comprised of 3 major components (Fig. S1b): spreading, reagent, and transparent layers. A top layer of TiO_2-cellulose acetate was designed for sample absorption and spreading. A middle reagent layer contained all reagents necessary for color development, which included glucose oxidase (*Aspergillus niger*, E.C. 1.1.3.4) 0.77 U/cm^2, peroxidase (horseradish root, E.C.1.11.1.7) 3.6 U/cm^2, 4-aminoantipyrine hydrochloride 0.11 mg/cm^2, and 1,7-dihydroxynaphthalene 67 μg/cm^2. The reagent layer also included appropriate buffers, surfactants, stabilizers, and binders. The bottom (transparent) layer was designed for support and enablement of detection only. Urea slides included five main components (Fig. S1d): spreading, reagent, semipermeable, indicator, and support layers. The reagent layer contained urease (jack beans, E.C.3.5.1.5) 1.2 U/cm^2 and buffering reagents to maintain pH 7.8 for the urease reaction. A semipermeable membrane under the reagent layer allows only ammonia to permeate, preventing transport of ammonium ions, which makes the urea slides specific to urea only, not the interfering ammonium in the blood. The indicator layer contained 0.26 mg/cm^2 of *N*-propyl-4-(2,6-dinitro-4-chlorobenzyl)-quinolonium ethane sulfonate as an ammonia indicator. The design of the spreading layer of the Vitros® slide ensures even distribution of sample in the horizontal plane. Non-proteinaceous components are passed through to the underlying reagent layer, where the enzymatic reactions occur.

The glucose slide color chemistry relies on Trinder reagents: peroxidase catalyzes oxidation of 4-aminoantipyrene and coupling of 1,7-dihydroxynapthalene to generate a dye with red color in proportion to glucose concentration [25] (Fig. S2). The urea assay color chemistry depends on the generation of ammonia from the urease catalyzed degradation of urea at pH 7.8. Permeation of ammonia through the semipermeable membrane allows it to react with the indicator [*N*-propyl-4-(2,6-dinitro-4-chlorobenzyl)-quinolonium ethane sulfonate] to generate a dye with grey-blue color in proportion to urea concentration (Fig. S3).

2.2. Collection of reflectance spectra with a spectrometer

Glucose and urea slides were brought to room temperature (23 ± 1 °C) from storage at −20 °C by allowing them to warm up to room temperature for one hour immediately prior to each experiment. D-(+)-glucose of 400 mg/dl and urea of 45 mg/dl (both from Sigma-Aldrich) were prepared with 10 mM PBS buffer (all buffer reagents from Mallinckrodt Baker, Inc.). For each slide, 10 μl of glucose or 5.5 μl of urea was applied to the center of the active areas. Slides were allowed to incubate at room temperature for 5 min for glucose and 20 min for urea to generate the colored products. An AvaSpec-2048 L spectrometer equipped with an AvaLight-DH-S-BAL Deuterium/Halogen continuum source, an RPH-1 Reflection Probe Holder, and AvaSoft© 7.6.0 software (Avantes) were used to measure the diffuse reflectance for the fully developed colored test slides in the wavelength range of 380 nm to 730 nm with the probe positioned 45° from the surface of the slides.

2.3. Collection of RGB values with a smartphone

The color of the test slides intensified with time and correlated directly to the concentration of the analyte in the test samples. RGB intensity values were measured and recorded via digital images collected using the built-in 5-megapixel iSight camera on an iPhone 4 (Apple Inc.) A simple homebuilt box with dimensions of 180 × 115 × 80 mm, painted flat black on its interior, was used to regulate light conditions during measurements. Inside the box, three bright LEDs were used as broad-spectrum light sources (5 mm, 13,850 K color temperature, 1800 mcd white LEDs, Super Bright LEDs Inc.). A commercially available mobile application designed for color matching, ColorAssist (FTLapps, Inc.), was used as a tool to separate and quantitate the color intensities of the digital images into red, green, and blue (RGB) channels at predetermined temporal endpoints (described below in Section 2.4). All of the experiments were carried out at room temperature (23 ± 1 °C).

2.4. Enzymatic kinetic and color stability studies of glucose and urea slides with a smartphone

Kinetic studies were conducted for selection of optimal incubation times for stable and reproducible color development and sensitive detection. Various concentrations of glucose and urea were added to the Vitros® slides before RGB values were collected with the iPhone 4 ColorAssist app over 60 min. The RGB values for fully developed glucose and urea slides were collected for long-term storage stability studies.

2.5. Development of calibration curves with a smartphone

Glucose and urea standards were prepared with a set of standard calibrator samples (Ortho Clinical Diag.) that mimic the matrix components in actual serum samples. Glucose and urea slides were prepared as described above, with 5 min of incubation for glucose and 20 min of incubation for urea at room temperature. RGB values were then measured using the iPhone and recorded. Green channel values were used to develop the calibration curves, as the green channel data was the most stable and reproducible. Five replicate experiments were carried out for both the glucose and urea assays.

2.6. Performance of the smartphone colorimetric method as compared to standard instruments

Canine whole blood samples were collected from five different dogs and tested at the College of Veterinary Medicine, Oregon State University.