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Measurement of Serum Phosphate Levels Using a Mobile Sensor

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Abstract

The measurement of serum phosphate concentration is crucial for patients with advanced chronic kidney disease (CKD) and those on maintenance dialysis, as abnormal phosphate levels may be associated with severe health risks. It is important to monitor serum phosphate levels on a regular basis in these patients; however, such measurements are generally limited to every 0.5-3 months, depending on the severity of CKD. This is due to the fact that serum phosphate measurements can only be performed at regular clinic visits, in addition to cost considerations. Here we present a portable and cost-effective point-of-care device capable of measuring serum phosphate levels using a single drop of blood (<60 μl). This is achieved by integrating a paper-based microfluidic platform with a custom-designed smartphone reader. This mobile sensor was tested on patients undergoing dialysis, where whole blood samples were acquired before starting the hemodialysis and during the three-hour treatment. This sampling during the hemodialysis, under patient consent, allowed us to test blood samples with a wide range of phosphate concentrations, and our results showed a strong correlation with the ground truth laboratory tests performed on the same patient samples (Pearson coefficient $r = 0.95$ and $p<0.001$). Our 3D-printed smartphone attachment weighs about 400 g and costs less than 80 USD, whereas the material cost for the disposable test is < 3.5 USD (under low volume manufacturing). This low-cost and easy-to-operate system can be used to measure serum phosphate levels at the point-of-care in about 45 min and can potentially be used on a daily basis by patients at home.

Graphical Abstract

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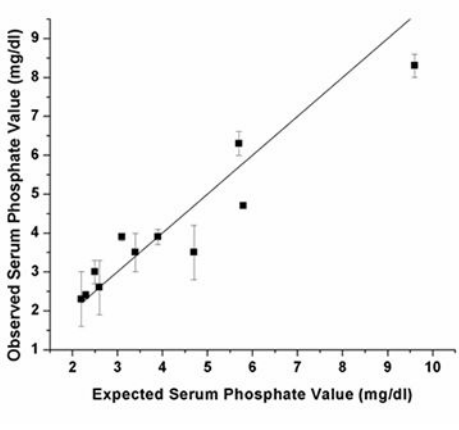
#Equal Contribution

Conflicts of interest

There are no conflicts to declare.



We report a portable and cost-effective point-of-care device capable of measuring serum phosphate levels using a single drop of blood.

Introduction

Phosphate is one of the most important analytes in blood as it is essential for multiple functions, including e.g., energy exchange, membrane transport, and intracellular signal transduction [1,2]. Phosphate ions are used by the body to carry out metabolic processes such as the production and storage of energy, buffering of blood, regulation of gene transcription, and enabling the transduction of signals that regulate pathways which affect organ functions in different endocrine systems[1]. The normal serum phosphate concentration ranges between 2.5 - 5.6 mg/dl in healthy adults, and values are age dependent in children. The maintenance of normal serum phosphate levels is regulated by a complex system that includes organ cross-talk between the intestines, kidney, bone, and parathyroid glands. Such interactions affect intestinal absorption, reabsorption and excretion by the kidneys, and the flux of phosphate between extracellular and skeletal pools[2]. Hypophosphatemia and hyperphosphatemia may give rise to a number of skeletal abnormalities, cardiovascular diseases, and impairments in muscular function. Humans with rare specific genetic mutations develop hypophosphatemia (hypophosphatemic rickets)[3] or hyperphosphatemia (i.e., tumoral calcinosis)[4].

However, the most common cause of elevated serum phosphate levels is chronic kidney disease (CKD). CKD is a significant problem that can affect both children and adults, and is characterized by progressive, irreversible deterioration of kidney function that ultimately leads to end stage renal disease (ESRD) and the need for dialysis and renal transplantation[5]. Several studies have demonstrated that disturbances in serum phosphate homeostasis increase when glomerular filtration rate (GFR) decreases below 60 ml/min/1.73m^2 [6]. As of 2013, more than 468,000 patients with ESRD were being treated with some form of dialysis treatment in the USA [7] . Several epidemiological studies have highlighted associations between serum phosphate levels and vascular calcifications, cardiovascular morbidity, and overall mortality—not only in patients treated with dialysis, but also in those with early stages of CKD[8,9,10,11,12,13]. In addition, studies in humans with normal renal function have

shown that even small increases in serum phosphate levels are associated with increased morbidity and mortality[9].It has also been established that phosphate induces vascular calcification and endothelial dysfunction; thus, there is clear evidence that phosphate promotes trans-differentiation of vascular smooth muscle cells to a chondro-osteoblast like phenotype[14]. Indeed, hyperphosphatemia is an independent risk factor for the progression of cardiovascular disease[9]. Currently, prevention and treatment of hyperphosphatemia with diet and phosphate binders is the cornerstone strategy recommended for patients with CKD [6]. Although enteral phosphate binders are effective, one of the major problems is poor compliance due to frequent administration (up to 3-5 times daily) and pill burden. Despite the overwhelming evidence of phosphate toxicity in CKD, serum phosphate levels are monitored only once or twice per month in dialysis patients and every 3-6 months in non-dialysis CKD patients[15].

Despite the emerging medical advances in dialysis technology in the 21st century and more than $22 billion per year in expenditures, the mortality rates of ESRD patients remain elevated. Therefore, ESRD remains an area where more effective treatment and monitoring technologies are critically needed. This is especially important for children and young adults that lack the traditional risk factors associated with cardiovascular disease. Thus, elevated serum phosphate levels represent an important health issue and a clear morbidity and mortality risk.

Currently, serum phosphate determinations are performed in laboratory settings that require bulky and expensive photometric and/or electro-chemiluminescence equipment [16,17]. There is a need to develop tools to empower patients and families to monitor serum phosphate levels at home, or in resource-limited settings, in order to promote close monitoring of phosphate levels in response to dietary changes, and to better inform phosphate binder management. Such an approach has been very successfully applied for the monitoring of serum glucose in patients with diabetes[18]. Indeed, the wide application of this home-based glucose sensing technology has markedly improved clinical outcomes in diabetes management. There is a strong clinical need for an analogous field-portable, reliable, and cost-effective serum phosphate sensing device that can potentially be used even at home.

To address this unmet medical need, we developed a portable and cost-effective phosphate sensing platform based on a smartphone with a custom-designed opto-mechanical attachment and an integrated paper-based microfluidic chip for measuring serum phosphate levels (see Figure 1). Such point-of-care (POC) devices based on mobile phones[19,20,21,22,23,24,25,26, 27] and optical imaging[28] have been used to detect and measure various biological agents[29], including albumin[30], herpes virus[31], Borrelia bacterial species (which cause Lyme disease[32]), Schistosoma haematobium[33], among others. Our phosphate sensing device requires a small volume of blood and a paper-based disposable chip is used for serum separation from blood. This serum is then tested using a colorimetric assay that is integrated into a custom designed attachment mounted onto the camera unit of the smartphone. The phosphate concentration in blood was quantified using a ratio-metric analysis and a calibration curve, which was validated using a standard laboratory-grade multi-analyte auto-analyzer. Each measurement, from the beginning to the end, takes ~45 min to complete and requires only ~ 20-50 μL of whole blood from the patient. To the best

of our knowledge, this is the first mobile POC sensor capable of measuring phosphate levels in serum. The device was further tested using blood samples taken from patients before and during hemodialysis sessions (as the serum phosphate levels decrease during the treatment), providing a strong correlation with the ground truth laboratory tests performed on the same samples.

Results and discussion

Design and optimization of the paper-based phosphate sensor

One of the crucial challenges in measuring serum samples for POC analysis is the difficulty in efficiently separating and collecting serum from whole blood in precise quantities. Our approach addresses this challenge by using a paper-based microfluidic chip as shown in Figure 1E, which can efficiently separate serum from small quantities of blood. This paper-based blood separation chip is composed of a sample pad, a red blood cell separation membrane, and a sample collection pad (Figure 1E). When a droplet of whole blood sample is placed onto the sample pad, it gets rapidly absorbed due to the hydrophilic nature and the capillary action of the pad. The sample then moves to a red blood cell separation membrane that is made of asymmetric polysulfone, designed to capture the serum components from whole blood. For our device, we used a GR grade membrane, which provides a plasma yield of > 80% and exhibits low non-specific binding[34], rendering it attractive for use in POC diagnostic devices[32,35]. The serum sample moves to the collection pad via the lateral flow based separation membrane. Eventually, when the collection pad is saturated, no further fluid flow is possible, and the flow stops. These engineered features allow for consistent collection of a fixed amount of serum from a single drop of whole blood (~ 25-50 μL)[36].

An important characteristic of our serum collection pad is its low cross-reactivity with the malachite green assay (see the Methods section for details). This assay contains malachite green dye and an acid-molybdate solution, where the presence of free orthophosphates leads to the formation of a phospho-molybdate complex, resulting in a colorimetric change[37]. We screened several paper materials such as nitrocellulose membranes and fiberglass-based conjugation pads as candidates for the collection pad (see Figure S1 and S2). Finally, Grade standard 14 conjugation pad[32] was selected as it was found to have the least reactivity with the malachite green assay. In order to precisely maintain the pressure between the separation pad and the collection pad, we designed a two-part holder, consisting of a serum separation part and the collection pad holder, as shown in Figure 1D. The serum separation part holds the serum separating chip, and the collection pad is attached to the pad holder. We use two magnets to maintain a uniform and constant pressure between the chip and the collection pad. A schematic of the entire serum separation device is shown in Figure 2. The volume of serum gathered using the collection pad was first characterized by creating a calibration curve using different volumes of serum as shown in Figure 3. We estimate that, on average, the collection pad can hold ~1.55 μL of serum, with an error of less than 10% (~0.15 μL).

Once the serum is collected in the collection pad, the pad holder is manually lowered into a sample well containing water, and the malachite green reagent is added to this well, as shown in Figure 2. The collection pad is passively incubated in this well for 30 min before being withdrawn. The pad holder is held in place during the entire incubation phase by an

adaptor. Upon the completion of the reaction, the adaptor is pulled out to release the pad holder, which is magnetically retracted back. For each serum phosphate measurement, two separate wells containing the malachite green reagent were used, with one serving as a control and the other for the serum sample. After interacting with the phosphate in the collected serum, there is a colorimetric change of the reagent. The ratio of the colorimetric signal change between the control and sample was used to determine the phosphate concentration using a mobile phone-based reader, discussed next. Each test was performed using two different volumes of water and reagent in order to cover a clinical dynamic range of up to ~10 mg/dL of serum phosphate concentration (see the Methods Section). Utilizing the 2-well cartridge facilitates a simple, easy-to-use and low-cost design for the reaction chamber as well as the readout.

Calibration and testing of the mobile phone-based assay reader

The assay quantification was performed automatically using a mobile phone-based reader that holds the well cartridge as well as the serum separator, as shown in Figure 1. After completion of the measurement, the serum separation device and the well cartridge containing the reagent need to be discarded and replaced with new ones. The optomechanical attachment, weighing ~400 g, is fitted with two different LEDs and a diffuser to ensure uniform light intensity at the sample and control wells.

We first created two different calibration curves with known amounts of phosphate using two different volumes of standard and reagent solutions (calibration-1: 50 μl water/standard + 100 μl reagent and calibration-2: 100μl water/standard + 200 μl reagent), as shown in Figure 4. The calibration curves were created by taking the ratio (R) of the intensity of the light transmitted through the sample well and the control well, and were used for the automated determination of the phosphate concentration in an unknown blood sample. The phosphate value is calculated based on the slope (S) and y-intercept (Y) of the linearly fitted calibration curve, i.e.,

$$Serum\ Phosphate = \frac{R - Y}{S} \times K \qquad \text{Eq. (1)}$$

where K is an empirical constant used to incorporate the dilution factor of the serum that is tested, and convert the concentration unit from μM to mg/dl. The first calibration curve, which is based on an assay utilizing 50 μl water and 100 μl reagent, enables the measurement of up to 4.5 mg/dl serum phosphate concentration. The second calibration curve is used to increase this range to 10 mg/dl by diluting the serum (i.e., utilizing 100 μl water and 200 μl reagent, see the Methods section). These two calibration curves have two separate K values associated with them. The parameters in Eq. (1) as well as the error in the serum volume in the collection pad were used to numerically estimate the overall error in our measurements. For example, an error of ~0.50 mg/dl is estimated based on the first calibration curve and ~0.98 mg/dl based on the second calibration curve for a serum phosphate concentration of 4.5 mg/dl. The lower and upper limit of detection (LOD) of the device is 0 mg/dl and 10 mg/dl, respectively, which are calculated based on the LOD of the malachite assay (0-50 μM).

Proof of concept experiments to test our mobile sensor were performed by measuring the serum phosphate levels from pediatric end-stage renal disease patients receiving hemodialysis treatments three times a week. Whole blood samples were obtained at baseline (just before starting the hemodialysis treatment) and at regular intervals during the three-hour treatment. During each hemodialysis treatment, whole blood was collected from the pre-dialyzer side of the hemodialysis circuit. Collecting blood at different time points during the hemodialysis session allowed us to assess blood samples with a wide range of phosphate concentrations, as serum phosphate is usually high in ESRD patients at the beginning of hemodialysis treatments, then decreases as the treatment proceeds[38] (Figure S3). Each whole blood sample was collected in heparin coated serum separator tubes and tested using our sensor (see the Methods Section). Approximately 40 μl blood was used per test and the tests were performed in triplicates (except x=5.8 mg/dl, was performed once due to limited sample volume). The rest of the whole blood sample was used to extract the serum via centrifugation and stored in −80 °C freezer for independent laboratory validation at a later time point. Serum phosphate levels of three patients measured at different time points using our mobile device (Figure 5 and Table S1) showed strong correlation with independent laboratory measurements with a Pearson correlation coefficient of $r = 0.95$ and $p<0.001$. A fourth patient was also tested while undergoing dialysis, however we observed a significant amount of precipitation in the reagent upon reacting with the serum. The reason for this particular observation is unclear, however this could have been due to e.g., an interference from a particular drug that the patient took, or high amount of serum proteins interfering with this assay.

It should be noted that the chemicals used for this assay has been shown to cause skin and eye irritation and therefore caution should be exercised during handling. After every use, the user will need to dispose the serum separation chip as well as the cartridges containing the malachite green assay and replace them with a new chip and cartridge. The attachment is designed in a way that would enable the user to easily change these components after every use. With future development, the malachite green assay can also be vacuum-sealed within test cartridges. The serum separation chips will also need to be sealed in order to protect them from moisture but can be stored at room temperature. In future work, other strategies such as dried malachite green absorbed on the pads can also be explored, which will eliminate the need for liquid chemicals, making it potentially easier for point-of-care applications.

Experimental

Serum Separation Chip:

The serum separation chip was constructed as a lateral flow device as shown in Figure 1E. A plastic adhesive backing was used to support the structure. A 2 mm hole was punched out on the plastic backing and covered with the sample pad. A 3 mm diameter circle of Fusion 5 (GE Healthcare Biosciences Corporation) was used as the sample pad. The GR Plasma Separation Membrane (4 mm x 8.75 mm, Pall Company), that acts as the filter, was placed directly on top of the sample pad ensuring no leakage from the sample pad sides. Additionally, a piece of 6 mm wide adhesive tape was used to completely seal the area

below the sample pad. A second piece of tape (2 mm wide) was used below the first one at a distance of ~ 2-3 mm to prevent any blood from leaking onto the collection pad. The tape and the membranes were precisely cut using a laser cutter.

Preparation of the Serum Separation Device:

A two-part device was 3D printed and used to hold the serum separation chip. The casing consists of two separable components. The lower half is the chip holder, and the upper half is the sample collector. A Grade standard 14 conjugation pad (GE Healthcare Biosciences Corporation) was used as the collection pad. Two magnets were used to maintain uniform contact between the collection pad and the plasma separation membrane. This ensures that there is a good flow of serum from the membrane to the collection pad, and that the collection pad can be fully saturated.

Serum Separation:

We tested several different blood volumes, ranging from 10 μl to 100 μl. The device worked consistently for a volume range of 10 μl to 60 μl. Thus, for our application, we used 40 μL of blood which was introduced onto the collection pad of the device. An average finger-prick yields about 25 μl of blood[36] and therefore we tested the collection efficiency with 20 μl and 40 μl of blood, observing similar results as shown in Figure S4. To ensure the complete saturation of the collection pad, we let the device sit for 10 min. This allowed the sample to flow through the membrane and for the collection pad to become completely saturated with serum. However, the serum separation often occurs more quickly, and we observed saturation of the collection pad within 5 min. After 10 min, the collection pad is manually lowered onto the well cartridge below it and set for 30 min (passive reaction time), using a manual attachment, for the reaction to complete. The volume of serum collected in the collection pad was determined by using a calibration curve (Figure 3). This calibration curve was created by recording the absorbance of the reagent (100 μL) upon interaction with a fixed amount of serum diluted in 50 μL water. These measurements were performed in a 96 well plate using a standard plate reader. The volume of serum in the collection pad was determined by comparing the absorbance due to the interaction of the serum-soaked collection pad in water and reagent, with the calibration curve shown in Figure 3.

Determination of Phosphate Concentration:

Following a 30-minute incubation, the collection device was removed by detaching the attachment. The colorimetric change was recorded using the cell phone camera as illustrated in Figure 1. The mobile phone reader has two 3D printed parts that hold the wells and the phone in place while imaging. Two battery-powered LEDs (Digikey, #475-1298-1-ND, peak wavelength: 624 nm, 12700 mcd, 30° viewing angle with a lens size of 2.6mm diameter) were used as the illumination source. Light from the LEDs passed through a paper diffuser, followed by a narrow channel to irradiate the wells placed at the end of the channel. The phone was placed at a distance of 5 cm from the sample to ensure optimal sensitivity. For our application, we used a Nokia Lumia phone, which was placed upside down in the holder so that the cell phone camera, lens, sample, and LEDs were properly aligned. The phone camera settings were manually set to 1/8000 exposure and infinity focus.

The analysis was performed using a combination of MATLAB, Image J as well as a smart phone application (app). The images were analyzed by first splitting the color channels and using the red channel. The centroids of the spots were first detected and the mean intensity of each spot was calculated using a small region of interest (200 by 200 pixels around the centroid), and the same was done for the control. The mean intensity of the spot corresponding to the sample was divided by the control mean intensity to obtain the intensity ratio. The assay was performed by placing the sample well on the right and the control well to its left. The mean intensity ratio was used to determine the phosphate concentration by using pre-prepared calibration curves. The 3D printed system encloses the camera; therefore, there is minimal interference from ambient light. The smartphone app is used to capture the image, which is then uploaded to a server, where the computation is performed using a MATLAB code, based on the algorithm described above. The calculated serum phosphate level is then displayed in mg/dl unit. The app can also be used to analyze an old test result/ image or images acquired by a different device at an earlier time point.

Colorimetric Phosphate Assay:

We used a malachite green assay (Bioassay Systems, DIPI-500) to determine the phosphate concentration of blood samples. DIPI-500 showed no interference with the selected paper materials, and additionally demonstrated the least interference with serum proteins. Two different protocols with DIPI 500 were used for this purpose. The collection pad was first put in either 50 μl or 100 μl water. Then, either 100 μl (with 50 μl water) or 200 μl (with 100 μl water) of the malachite green assay was added, and the mixture was allowed to incubate for 30 minutes. The absorbance was measured using our cell phone-based reader and cross-validated using a standard plate reader. The initial measurements were performed using 50 μl water (with 100 μl reagent) as it is more sensitive at lower serum phosphate concentrations; however, the upper limit of detection of the assay with this ratio is 5 mg/dl. If the result of the first test indicated a value greater than 4 mg/dl, then we repeated the measurements using 100 μl water (with 200 μl reagent). If the second test resulted in a value <4.5 mg/dl, then the first measurement result with 50 μl water was recorded. Otherwise the result of the second test with 100 μl water was recorded. For serum phosphate values of <4.5 mg/dl, we preferred the assay with 100 μl reagent (50 μl water) based on the calibration curve-1 as it has a lower error compared to the second calibration curve. The upper limit of detection of the specific malachite green assay we used is 50 μM phosphate. Thus, upon diluting the serum (1.55 μl) in water (50 μl), a dilution of ~32 fold is created, which sets the upper limit of detection to ~5 mg/dl of phosphate concentration in serum. However, by using 100 μl of water and diluting 1.55 μl of serum in it, we increase the dilution to ~64 fold, thus enabling serum phosphate measurements of up to ~10 mg/dl concentration.

Preparation of the Calibration Curves:

The calibration curves were prepared using phosphate standards. The standards were prepared from a 1 mM phosphate solution. For our application, we first prepared a 50 μM solution and then used it to generate the following concentrations: 5, 10, 15, 20, 25, 30, 35, 40, and 45 μM, in addition to the control sample. The accuracy of the dilution was checked by comparing the 30 μM sample, that we prepared via serial dilution, with commercially available samples of equal concentration. We observed a slight difference in absorbance

between the two 30 μM samples (commercial vs. the one we prepared via serial dilution), and this difference was used to accordingly adjust the other points on the calibration curve. Two different calibration curves were prepared by adding 100 μl reagent to 50 μl phosphate standard (Calibration-1) and 200 μl reagent to 100 μl phosphate standard (Calibration-2). These calibration curves were prepared by placing the sample well on the right and the control well to its left. The error bars associated with pipetting error in Figure 4 can be significantly reduced by using automated pipetting.

Validation of the Serum Phosphate Measurements Using a Laboratory Instrument:

To provide ground truth comparison measurements, the serum was separated from whole blood for validation studies by centrifuging blood at 10,000g for 10 min. The resultant serum was removed from the serum separator tube and aliquoted into new tubes. These serum samples were stored at −80 °C. The serum samples were then delivered to a central chemistry lab, where the serum phosphate concentration was measured using an Alfa-Wasserman ACE® Alera Systems analyzer. The correlation between serum phosphate concentrations measured by the central chemistry lab and by our mobile sensor was assessed using SigmaPlot 12.5 (San Jose, CA).

Patient recruitment and consent:

Patients with end-stage renal disease were recruited from the Davita/Century City dialysis unit. This study was approved by UCLA Human Subjects Protection Committee, (IRB # 10-000886) and informed consent was obtained from each patient. Two patients signed the consent themselves and two others were signed by their parents/guardian because they were < 18 years.

Conclusions

We presented a cost-effective point-of-care device capable of accurately measuring serum phosphate concentrations by combining a paper-based microfluidic chip and a smartphone-based reader. A small quantity of blood is loaded onto the lateral flow microfluidic chip that separates the serum from whole blood. A collection pad is used to collect the serum sample, which is then transferred to a well containing malachite green reagent. The colorimetric change of the solution is recorded using a smartphone and is used to determine the serum phosphate concentration. This device was tested using clinical samples obtained while the patients were undergoing dialysis session. Our results demonstrated a strong correlation between the independent laboratory measurements and our mobile sensor readings ($r = 0.95$, $p<0.001$), demonstrating the ability of this cost-effective POC device to accurately measure serum phosphate concentrations. This mobile sensor might benefit a vast number of individuals, including patients with CKD/ESRD, as well as others who need frequent monitoring of serum phosphate levels.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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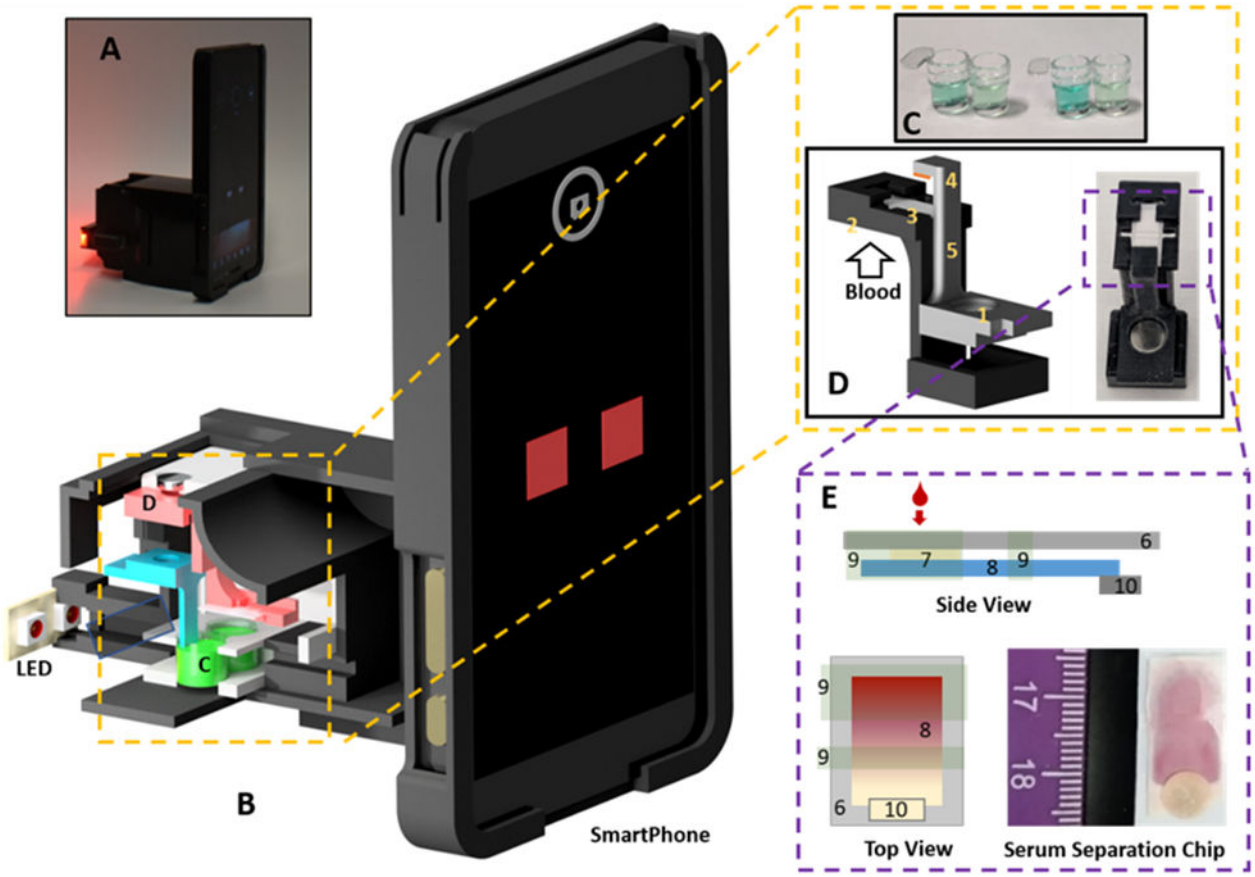

Figure 1.

(A) Photograph of the device along with the smartphone. (B) Schematic of the mobile attachment depicting all the components: smartphone, smartphone attachment consisting of two LEDs followed by a diffuser, Serum separation device and the well cartridge containing the assay (inset). (C) The two wells (serum -left and control-right) following 30 min incubation. (D) Schematic of the two-part holder for the chip, consisting of the blood separation unit and the serum collector part. The components of the holder are as follows: 1. Magnet, 2. Serum separation chip holder, 3. Serum separation chip, 4. Collection Pad, 5. Collection pad holder. A photograph of the actual component is placed next to the schematic. (E) The schematic of the serum separation chip. The labels are as follows: 6. Plastic adhesive backing, 7. Sample pad (Fusion 5), 8. Membrane (VPS GF/GR), 9. Adhesive tape, 10. Collection pad. A photograph of the serum separation using the paper based chip is also shown on the right.

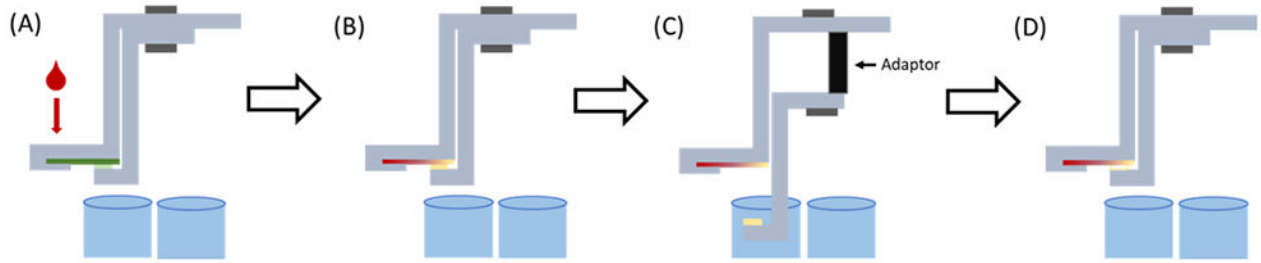

Figure 2:

Schematic depicting the step by step process of serum separation and assay. (a) Deposition of the blood on the chip, (b) Serum separation and collection in the collection pad, (c) The collection pad is submerged into a well containing water and reagents. The collection pad is lowered manually but is held in place using an adaptor. (d) Following the incubation period, the adaptor is manually removed and the collection unit gets retracted back due to a magnetic force.

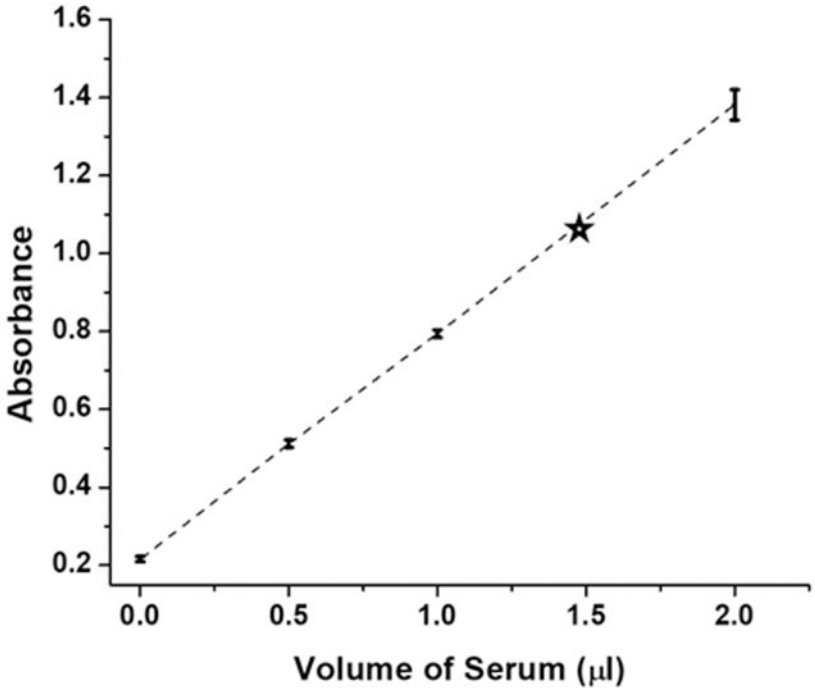

Figure 3.

Determination of serum volume collected in the collection pad. A calibration curve was created by adding different amounts of serum (in water) with the reagent. The star denotes the absorption from reagent upon interaction with the collection pad, thus indicating its volume. The measurements were performed in triplicates and the standard deviation was calculated based on the individual measurements.

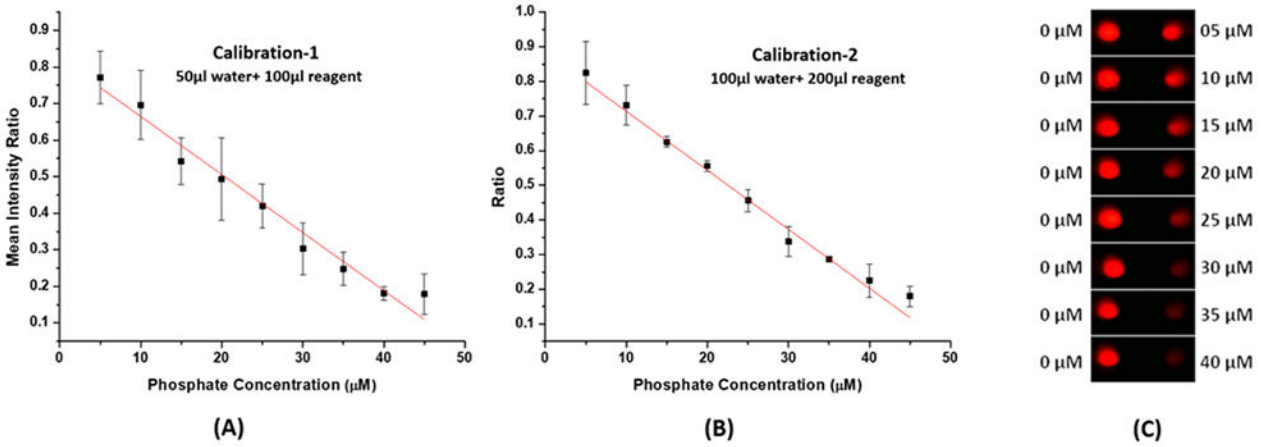

Figure 4.

Calibration curves using phosphate standards and two different amounts of reagents. (A) Calibration-1 was performed by adding 100 µl reagent to 50 µl water/phosphate standard. (B) Calibration-2 was performed by adding 200 µl reagent to 100 µl water/phosphate standard. The measurements were performed in triplicates. (C) A set of images acquired using the smartphone for various phosphate concentrations. The spots on the left correspond to the control wells and the spots on the right correspond to the phosphate buffer at different concentrations added to the assay.

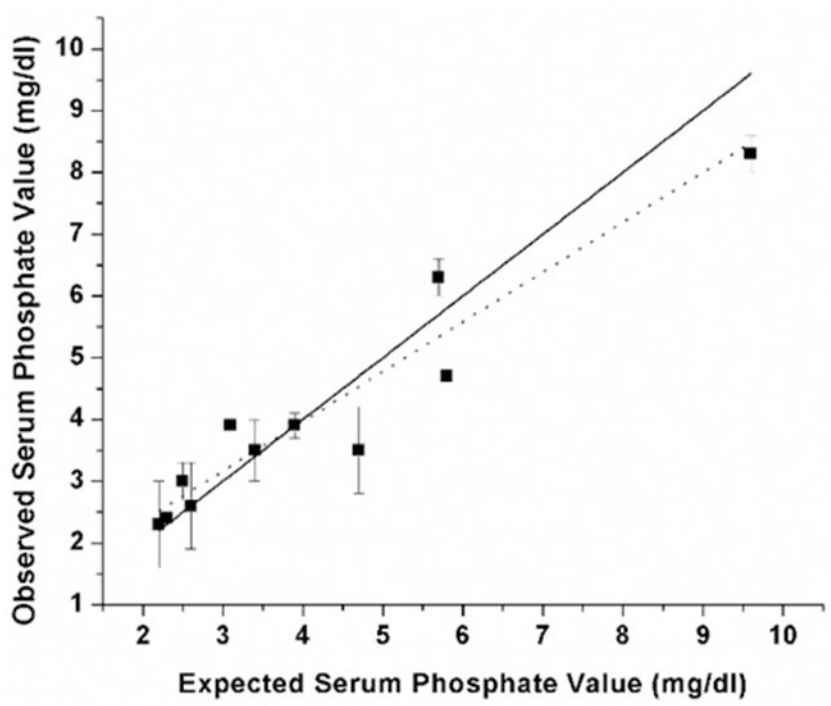

Figure 5.

Patient testing results. Comparison of our mobile sensor serum phosphate measurement results against the results of a laboratory instrument (Alfa-Wasserman ACE® Alera Systems analyzer), which yields r=0.95; $P < 0.001$. The solid line shows $y=x$ (i.e., the true value). A linear fit to the experimental data is also shown with a dotted line, with an R-squared value of 0.91.