Paper-based diagnostic devices for clinical paraquat poisoning diagnosis

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This article unveils the development of a paper-based analytical device designed to rapidly detect and clinically diagnose paraquat (PQ) poisoning. Using wax printing technology, we fabricated a PQ detection device by patterning hydrophobic boundaries on paper. This PQ detection device employs a colorimetric sodium dithionite assay or an ascorbic acid assay to indicate the PQ level in a buffer system or in a human serum system in 10 min. In this test, colorimetric changes, blue in color, were observable with the naked eye. By curve fitting models of sodium dithionite and ascorbic acid assays in normal human serum, we evaluated serum PQ levels for five PQ-poisoned patients before hemoperfusion (HP) treatment and one PQ-poisoned patient after HP treatment. As evidenced by similar detection outcomes, the analytical performance of our device can compete with that of the highest clinical standard, i.e., spectrophotometry, with less complicated sample preparation and with more rapid results. Accordingly, we believe that our rapid PQ detection can benefit physicians determining timely treatment strategies for PQ-poisoned patients once they are taken to hospitals, and that this approach will increase survival rates. Published by AIP Publishing. [<http://dx.doi.org/10.1063/1.4953257>]

INTRODUCTION

Paraquat (PQ) is a commonly used herbicide around the world, especially in developing countries, because it is a low-cost and readily accessible chemical.¹ PQ has been prohibited in most industrialized countries, but PQ poisoning caused by intentional ingestion or accidental exposure remains a major cause of mortality in less-developed countries.^{2,3} PQ is a highly toxic agent (mortality rate ~60%–80%) and has a high volume of distribution in biological bodies, which gives rise to its easy accumulation in multiple organs.^{4–6} PQ-poisoned patients die from multiple organ failure within several hours to days after ingestion of 40 ml of a 24% PQ solution.⁷ PQ-poisoned patients whose urine PQ concentrations 24 h after ingestion ranged from 25 to 50 ppm (as well as those with poisoning over 50 ppm) experienced an 88.9% mortality rate due to the lack of a proper antidote.⁸ At present, physicians treat PQ-poisoned patients on the spot with a standard PQ detoxification protocol in accordance with the detection results from a urine sodium dithionite assay and PQ poisoning symptoms.^{9–11} However, a urine sodium dithionite assay cannot accurately reflect PQ poisoning prognosis in patients. Time elapsed to treatment is significantly associated with survival rate; PQ-poisoned patients who accepted repeated pulse therapy with early hemoperfusion (HP) within 5 h post PQ ingestion had a 57.1% survival rate.³

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Serum/plasma PQ level has been considered a predictor to assess the prognosis of PQ poisoning.^{12,13} When plasma PQ concentration did not exceed 2 ppm at 10 h post poisoning, PQ-poisoned patients were more likely to recover.¹⁴ Note, one study proposed that patients with blood PQ levels of 2.0 ppm after 10–12 h ingestion had a 100% mortality rate.¹⁵ Various analytical methods to quantify PQ level in serum or plasma have been proposed, including high performance liquid chromatography (HPLC)/mass spectrometry (MS), gas chromatography (GC)/MS, capillary electrophoresis, enzyme-linked immunosorbent assay (ELISA), and spectrophotometry coupled with a sodium dithionite assay.^{12,15–19} Most of them, however, are time consuming and require complicated operational settings and experimental preparations.

Recently, paper-based analytical devices (PADs) have been regarded as potential translational medicine platforms for disease diagnostics.^{20–24} Paper is low-cost, ubiquitous, and easily disposed of by incineration. It is also lightweight, highly compatible with current technologies, possesses natural capillary action, and has a high surface area to volume ratio, which contributes to the development of affordable and user-friendly PADs for rapid biochemical analysis.^{25–27} Ample fabrication techniques exist for the creation of PADs, including printing with polymeric materials or waxes, inkjet etching, laser cutting, and other techniques.^{28–33} Moreover, PADs have been conjugated with diverse analytical technologies (e.g., colorimetry, chemiluminescence, electrochemical analysis) to detect a wide range of analytes in humans and in the environment.^{34–37} The strengths of PADs have been exploited as powerful analytical tools for a variety of diagnostic intents including, but not limited to the following: (1) a multiplexed electrochemical platform to detect human immunodeficiency virus (HIV) and hepatitis C virus (HCV) with limits of detection (LODs) for HIV and HCV assays of 300 pg/ml and 750 pg/ml, respectively; and (2) a three-dimensional detection sensor with a linear response for nitrite concentrations of 0–11.5 ppm and oxalate concentrations of 0–1000 mg/100 ml.^{38,39} Additionally, paper-based hybrid systems have been presented in response to hard flow control challenges and assay observation that suffers from opaque material properties.^{40,41} Nowadays, the development of simple pesticide biosensors mainly focuses on the quantification of organophosphorus or carbamate compounds in food or water because both pesticides are widely used in agriculture.^{42–44} PADs as pesticide detection devices may use photoelectrochemical or colorimetric assay platforms to indicate the presence of organophosphorus or carbamate pesticides to secure food quality.^{45–47} Notably, we, and others, have developed PADs to analyze serum organophosphate level for organophosphate poisoning in patients.⁴⁸ To the best of our knowledge, few previous studies have examined the use of PADs for clinically diagnostic PQ detection. Herein, we demonstrate the development of a PAD employing a sodium dithionite assay or ascorbic acid assay to determine serum PQ levels of PQ-poisoned patients in only 10 min.

MATERIALS AND METHODS

Chemicals

Paraquat dichloride hydrate (Sigma Aldrich, St. Louis, MO), sodium hydroxide (NaOH) (Sigma Aldrich, St. Louis, MO), sodium dithionite (85%, Sigma Aldrich, St. Louis, MO), phosphate buffered saline (PBS) (tablet, 85%, Sigma Aldrich, St. Louis, MO), ascorbic acid (Sigma Aldrich, St. Louis, MO), Whatman chromatography paper, 3 MM Chr sheets (GE Healthcare Life Sciences; No. 3030-917), Whatman qualitative filter paper, No. 1 (GE Healthcare Life Sciences; No. 1001-150).

Fabrication of PADs

Using Microsoft PowerPoint software as a drawing tool, we designed a 96-well microzone plate pattern in an 8 × 12 circle array (the diameter of each circle was 0.4 cm). To fabricate paper-based 96-well microzone plates, we printed the design on Whatman qualitative filter paper No. 1 (for sodium dithionite assay) and Whatman chromatography paper (for ascorbic acid assay) using a commercially available wax printer (Xerox Phaser 8560N color printer).

Afterwards, we used an oven (105 °C for 5 min) to heat the wax-printed microzone plates so that molten wax diffused into and completely through the paper to the other side, thus forming hydrophobic boundaries that could act as reaction wells, i.e., detection zones for our assays.

Colorimetric assays

In preparation for our sodium dithionite assay, we applied 5 μ l of 5 N NaOH and 2 μ l of 20% (w/v) sodium dithionite to the detection zones of a 96-well plate that was suspended (flat) in the air (note, liquids could not dry out in this experiment), and then immediately added 8 μ l of PQ solution in PBS/serum or patient serum. We then waited 10 min for the chemical reaction to complete under ambient conditions. Detection results were subsequently preserved with a digital camera (EOS 5D Mark III, Canon, Japan) and then analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

For our ascorbic acid assay, we applied 5 μ l of 5 N NaOH and 6 μ l of 5% (w/v) ascorbic acid to detection zones of a 96-well plate that was suspended (flat) in the air and waited 20 min for these liquid reagents to dry under ambient conditions. Subsequently, we added 8 μ l of PQ solution in PBS/serum or patient serum and waited 10 min for the chemical reaction to complete at ambient conditions (note, there is no need to wash in this assay protocol). Detection results were preserved with a digital camera and then analyzed using ImageJ software.

Image analysis

We used ImageJ software, downloaded from the National Institutes of Health, to analyze the RGB color values of each dithionite assay image result; processed mean intensity values were introduced into Delta RGB analysis ([supplementary material](#), Fig. S1). We also used ImageJ software to obtain the mean intensity red values for each ascorbic acid assay image result, subtracting original background values to diminish background noise and analytical variation.

Statistical analysis

Student's t-test was used to evaluate detection results with standard values from clinic reports. A value of $p < 0.05$ was considered statistically significant.

Serum sample collection

Clinical serum samples were collected at Chang Gung Memorial Hospital, Linkou Medical Center, Taiwan. Five serum samples were collected from each individual patient.

RESULTS AND DISCUSSION

Design of PADs for PQ detection

A schematic diagram of our PAD design is shown in Figure 1. In this study, we leveraged wax printing technology to fabricate 96-well plates as the format for our PQ detection device. This format facilitated multiple screening tests to investigate reaction conditions for PQ detection assays as we developed them (Fig. 1(a)). Reagents, including sodium dithionite, ascorbic acid, and sodium hydroxide were immobilized in paper, which is naturally absorptive (Fig. 1(b)). Colorimetric PQ detection was achievable because of blue radical ion formation in the presence of sodium dithionite or ascorbic acid in alkaline solutions.⁴⁹

Analytical performance of PQ detection assays in buffer and normal human serum systems

Before clinical validation, we established standard curves for sodium dithionite and ascorbic acid assays at five different PQ standard solution concentrations (i.e., 0, 5, 10, 25, and 50 ppm) in normal human serum (Fig. 2) and PBS ([supplementary material](#), Fig. S2). We employed chromatography paper and filter paper for sodium dithionite and ascorbic acid assays,

FIG. 1. Schematic diagram of our PQ detection device. (a) Fabrication of our PQ detection device, which is based on wax printing technology. (b) Detection reagents for our sodium dithionite assay or ascorbic acid assay were applied to each paper well using a pipet. Colorimetric responses (blue color) were correlated with PQ solution levels.

respectively, because these combinations contributed to better colorimetric attributions (paper screening data not shown). Figure 2 shows the results of our sodium dithionite and ascorbic acid assays in normal human serum. The LODs for sodium dithionite and ascorbic acid assays in normal human serum were 13.80 and 3.86 ppm, respectively, which was determined by the signal results of control groups plus three-fold standard deviations in linear regression models formed in the detection range from 0 to 25 ppm. Other than the influence of environmental light, the disappointing LOD results from our ascorbic acid assay in PBS may be due to the fact that the ascorbic acid assay did not produce noticeable color changes at different PQ concentrations in our buffer system (supplementary material, Fig. S2(b)). However, the color changes were relatively easy to observe when using our ascorbic acid assay in serum at different PQ concentrations. It is possible that original serum color contributed to the discrepancy due to the coupling effect of yellow serum and blue reaction products. By contrast, the coupling effect had only a minor influence on our sodium dithionite assay in serum system, which may be attributable to assay characteristics (e.g., reaction duration, assay preparation).

Quadratic models of our dithionite and ascorbic acid assays were used to investigate serum PQ levels in PQ-poisoned patients. Although the LODs of both assays in PBS and normal human serum systems were relatively higher than 2 ppm,¹⁵ both assays produced similar results when compared to the highest clinical standard method (spectrophotometry) for clinical PQ poisoning diagnosis (Tables I and II) despite the distinctive colors of serum from each patient.

Evaluation of serum PQ levels for clinical PQ poisoning diagnosis using PADs

Table I shows the comparative (our device/spectrophotometry (clinical reports)) serum PQ detection results from four PQ-poisoned patients before hemoperfusion (HP) treatment. Table II shows the comparative serum PQ detection results from a PQ-poisoned patient before and after HP treatment. Serum samples from five patients were obtained when they were taken to the hospital.

FIG. 2. PADs for PQ detection in normal human serum system. (a) We conducted a sodium dithionite assay using normal human serum and created a calibration curve under several concentrations—0, 5, 10, 25, and 50 ppm. ($N = 10$; mean intensity $\pm$ S.D.) (b) We conducted an ascorbic acid assay using normal human serum system and created a calibration curve under several concentrations—0, 5, 10, 25, and 50 ppm. ($N = 10$; mean intensity $\pm$ S.D.)

We used quadratic models of our dithionite and ascorbic acid assays in normal human serum (Fig. 2) to assess serum PQ levels for PQ-poisoned patients. The relative standard deviation (RSD) when measuring dithionite assay outcome was between 0.58% and 4.01% and that for ascorbic acid was between 1.37% and 8.50% (supplementary material, Table S1), which indicates that our device could analyze serum PQ level in a reliable and accurate manner. Figure 3 illustrates the linear regression analysis of PQ detection results by our device and by spectrophotometry, which indicates that sodium dithionite and ascorbic acid assays results for PQ analysis using our paper device were comparable to those achievable with spectrophotometry. We again note that the turnaround time for clinical results reporting using spectrophotometry is approximately 24 h, and, according to previous animal and clinical studies, the index of time elapsed to treatment is a critical rescue factor for PQ-poisoned individuals.^{3,6} Our device efficiently lowers PQ detection time down to only 10 min without the need for sophisticated training or expensive equipment, thus permitting physicians to diagnose patients in a timely, potentially life-saving, manner.

TABLE I. PQ detection results from four PQ-poisoned patients before HP treatment analyzed by our detection methods and by spectrophotometry, which is considered the highest clinical standard. ($N = 3$; mean intensity $\pm$ S.D.; sodium dithionite assay: $P < 0.83$, ascorbic acid assay: $P < 0.90$.)

	Patient 1	Patient 2	Patient 3	Patient 4
Gender	Female	Male	Male	Male
Age (years)	34	58	50	41
Poisoning source	Intentional ingestion	Intentional ingestion	Intentional ingestion	Accidental exposure
Time elapsed from ingestion to arrival at ER (hours)	1.5	N/A	6.5	2
Time elapsed from ingestion to the beginning of HP (hours)	25	N/A	8.5	4.3
Sodium dithionite assay (ppm)	3.31 ± 1.34	25.71 ± 1.78	1.89 ± 1.14	3.21 ± 1.04
Ascorbic acid assay (ppm)	3.15 ± 0.68	23.93 ± 0.61	1.92 ± 0.24	2.29 ± 0.24
Spectrophotometry (ppm)	2.69	22.08	1.75	2.37
Urine sodium dithionite test (ppm)	>50	>50	>50	>50

TABLE II. PQ detection results of a PQ-poisoned patient before and after HP treatment analyzed by our detection methods and by spectrophotometry, which is considered the highest clinical standard. ($N = 3$; mean intensity $\pm$ S.D.)

	Patient 5	Patient 5 (after HP treatment)
Gender	Male	Male
Age (years)	36	36
Poisoning source	Intentional ingestion	Intentional ingestion
Time elapsed from ingestion to arrival at ER (hours)	N/A	N/A
Time elapsed from ingestion to the beginning of HP (hours)	N/A	N/A
Sodium dithionite assay (ppm)	11.39 ± 2.29	1.10 ± 0.23
Ascorbic acid assay (ppm)	11.32 ± 0.24	0.78 ± 0.21
Spectrophotometry (ppm)	10	0.49
Urine sodium dithionite test (ppm)	>50	25

FIG. 3. Linear regression analysis of PQ detection results by our device and spectrophotometry (sodium dithionite assay: $R^2 = 0.99$; ascorbic acid assay: $R^2 = 0.99$).

Although the sensitivity and LODs in both assays in our current procedures were not ideal, we believe that this weakness can be ameliorated by further optimization or the selection of other detection platforms, including the use of vertical flow-based devices. Optimization may include developing fine detection protocols to provide advantageous color attributes or implementing a specific image recording device to collect colorimetric results in a closed system without environmental light interference. Expanding upon these efforts could very well lead to the functional development of a user-friendly PQ analytical device for critical care medicine. Further, this potential platform, which could be coupled with telemedicine ([supplementary material](#), Fig. S3), would allow users to assay small patient sample volumes taken directly from the patient with lancing devices and no pipetting equipment. In the future, hybrid devices based on this research may provide the medical diagnostic field with viable alternate detection platforms that carry three distinct advantages: (i) accurate fluid control that avoids pipetting errors; (ii) precise channel design; and (iii) ease of implementation for multiple testing.⁵⁰

CONCLUSIONS

We successfully demonstrated the development of a PAD for rapid PQ poisoning detection. While simplistic in design, quantifiable colorimetric results were rapidly and easily obtained.

This device is demonstrably efficient and accurate compared to spectrophotometry (the highest clinical standard) but can save time, money, and patient lives.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) regarding the Delta RGB calculation (Fig. S1), PADs for PQ detection in buffer system (Fig. S2), diagram of a potential PQ detection device (Fig. S3), and relative standard deviation (RSD) for measuring outcomes of sodium dithionite and ascorbic acid assays (Table S1).

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